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(54) Title: MAMMALIAN CHEMOKINES

(57) Abstract

Novel mouse and human CC and CXC chemokines, reagents related thereto including purified proteins, specific antibodies and nucleic acids encoding these chemokines are provided. Also provided are methods of making and using said reagents and diagnostic kits.

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MAMMALIAN CHEMOKINES

FIELD OF THE INVENTION

The present invention contemplates compositions related to proteins which function in controlling development, differentiation, trafficking, and physiology of mammalian cells, e.g., cells of a mammalian immune system. In particular, it provides proteins which regulate or evidence development, differentiation, and function of various cell types, including hematopoietic cells.

BACKGROUND OF THE INVENTION

The circulating component of the mammalian circulatory system comprises various cell types, including red and white blood cells of the erythroid and myeloid cell lineages. See, e.g., Rapaport (1987) Introduction to Hematology (2d ed.) Lippincott, Philadelphia, PA; Jandl (1987) Blood: Textbook of Hematology, Little, Brown and Co., Boston, MA.; and Paul (ed.) (1993) Fundamental Immunology (3d ed.) Raven Press, N.Y.

For some time, it has been known that the mammalian immune response is based on a series of complex cellular interactions, called the "immune network." Recent research has provided new insights into the inner workings of this network. While it remains clear that much of the response does, in fact, revolve around the network-like interactions of lymphocytes, macrophages, granulocytes, and other cells, immunologists now generally hold the opinion that soluble proteins, known as lymphokines, cytokines, or monokines, play a critical role in controlling these cellular interactions. Thus, there is considerable interest in the isolation, characterization, and mechanisms of action of cell modulatory factors, an understanding of which should lead to significant advancements in the diagnosis and therapy

of numerous medical abnormalities, e.g., immune system and other disorders.

Lymphokines apparently mediate cellular activities in a variety of ways. They have been shown to support the proliferation, growth, and differentiation of the pluripotential hematopoietic stem cells into vast numbers of progenitors comprising diverse cellular lineages making up a complex immune system. These interactions between the cellular components are necessary for a healthy immune response. These different cellular lineages often respond in a different manner when lymphokines are administered in conjunction with other agents.

The chemokines are a large and diverse superfamily of proteins. The superfamily is subdivided into two classical branches, based upon whether the first two cysteines in the chemokine motif are adjacent (termed the "C-C" branch), or spaced by an intervening residue ("C-X-C"). A more recently identified branch of chemokines lacks two cysteines in the corresponding motif, and is represented by the chemokines known as lymphotactins. Another recently identified branch has three intervening residues between the two cysteines, e.g., CX3C chemokines. See, e.g., Schall and Bacon (1994) Current Opinion in Immunology 6:865-873; and Bacon and Schall (1996) Int. Arch. Allergy & Immunol. 109:97-109.

Many factors have been identified which influence the differentiation process of precursor cells, or regulate the physiology or migration properties of specific cell types. These observations indicate that other factors exist whose functions in immune function were heretofore unrecognized. These factors provide for biological activities whose spectra of effects may be distinct from known differentiation or activation factors. The absence of knowledge about the structural, biological, and physiological properties of the factors which regulate cell physiology in vivo prevents the modulation of the effects of such factors. Thus, medical conditions where regulation of the development or

physiology of relevant cells is required remains unmanageable.

SUMMARY OF THE INVENTION

The present invention reveals the existence of previously unknown chemokine-motif containing molecules which are hereby designated mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine. Based on sequence analysis of the chemokine protein sequences described below, it is apparent that mpf4, mCTAP3, and Chr19kine belong to the CXC chemokine family, and m6Ckine and h6Ckine belong to the CC chemokine family.

The present invention provides a composition of matter selected from a composition comprising an antigen binding site from an antibody which specifically binds to mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine; an expression vector encoding mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine or fragment thereof; a substantially pure protein which is specifically recognized by the respective antigen binding site; and a substantially pure mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine or peptide thereof, or fusion protein comprising a 30 amino acid fragment of mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine sequence. Also provided are methods for making and using said reagents.

In the antigen binding site containing embodiments, the antigen binding site may be: specifically immunoreactive with a mature protein selected from the group consisting of the polypeptides of SEQ ID NO: 2, 4, 6, 8, or 10; raised against a purified or recombinantly produced mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine; in a monoclonal antibody, Fab, or F(ab)2; or in a labeled antibody. In certain embodiments; the antigen binding site is detected in a biological sample by a method of: contacting a binding agent having an affinity for the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein with the biological sample;

incubating the binding agent with the biological sample to form a binding agent:mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein complex; and detecting the complex. In a preferred embodiment, the biological sample is human or mouse, and the binding agent is an antibody.

A kit embodiment is provided possessing a compound, described above, with either instructional material for the use of the compound; or a compartment into which the compound is segregated.

A nucleic acid embodiment of the invention includes an expression vector encoding a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein, wherein the protein specifically binds an antibody generated against an immunogen selected from the mature polypeptide portions of SEQ ID NO: 2, 4, 6, 8, or 10, more particularly a natural protein. The vector may: encode a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine polypeptide with complete sequence identity to a naturally occurring mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein; encode a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein comprising sequence selected from the polypeptides of SEQ ID NO: 2, 4, 6, 8, or 10, and particularly a natural protein; or comprise sequence selected from the nucleic acids of SEQ ID NO: 1, 3, 5, or 7. In other embodiments, the vector is capable of selectively hybridizing to a nucleic acid encoding a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein, e.g., a mature protein coding segment of SEQ ID NO: 1, 3, 5, or 7. In various preferred embodiments, the isolated nucleic acid is detected in a biological sample by a method of: contacting a biological sample with a nucleic acid probe capable of selectively hybridizing to the nucleic acid; incubating the nucleic acid probe with the biological sample to form a hybrid of the nucleic acid probe with complementary nucleic acid sequences present in the biological sample; and determining the extent of hybridization of the nucleic acid probe to the

complementary nucleic acid sequences. In such method, preferably the nucleic acid probe is capable of hybridizing to a nucleic acid encoding a protein consisting of the polypeptides of SEQ ID NO: 2, 4, 6, 8, or 10. Also provided are methods of making an expression vector or making protein comprising construction or expression of a vector encompassing said nucleic acids.

In various embodiments, the isolated mpf4, mCTAP3, m6Ckine is from a mouse; the isolated h6Ckine is from a human; consists of a polypeptide comprising sequence from SEQ ID NO: 2, 4, 6, 8, or 10; recombinantly produced, or a naturally occurring protein. Also provided are fusion proteins comprising a sequence of SEQ ID NO: 2, 4, 6, 8, or 10 and/or a sequence of another cytokine or chemokine.

The present invention also embraces a cell transfected with the nucleic acid encoding a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine, e.g., where the nucleic acid has SEQ ID NO: 1, 3, 5, or 7. The cell may be either prokaryote or eukaryote.

The invention also provides a method of modulating physiology or development of a cell by contacting the cell with a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine, or an antagonist, e.g., neutralizing antibody, of the chemokine. In preferred embodiments, the physiology is attraction of a cell which possesses a natural receptor for the chemokine.

DETAILED DESCRIPTION

I. General

The present invention provides mouse and human DNA sequences encoding mammalian proteins which exhibit structural properties or motifs characteristic of a cytokine or chemokine. For a review of the chemokine family, see, e.g., Lodi, et al. (1994) Science 263:1762-1767; Gronenborn and Clore (1991) Protein Engineering 4:263-269; Miller and Kranger (1992) Proc. Nat'l Acad. Sci. USA 89:2950-2954; Matsushima and Oppenheim (1989) Cytokine 1:2-13; Stoeckle and Baker (1990) New Biol.

2:313-323; Oppenheim, et al. (1991) Ann. Rev. Immunol. 9:617-648; Schall (1991) Cytokine 3:165-183; and The Cytokine Handbook Academic Press, NY.

The novel cytokines described herein are designated mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine. The descriptions below are directed, for exemplary purposes, to mammalian embodiments, e.g., human and mouse, but are likewise applicable to related embodiments from other, e.g., natural, sources. These sources should include various vertebrates, typically warm blooded animals, e.g., birds and mammals, particularly domestic animals, and primates.

The nucleic acid and amino acid sequences of the novel Mouse mpf4 chemokine are provided in SEQ ID NO: 1 and SEQ ID NO: 2, respectively. The mpf4 coding sequence begins at base 141 and ends at base 458, the CXC motif is at amino acid residues 5-7. Absence of an ELR motif immediately preceding the CXC suggests likely anti-inflammatory properties. A signal sequence is indicated, but based upon related genes, variable forms may be produced by different cell types.

The nucleic acid and amino acid sequences of the novel mouse CTAP3 (mCTAP3) are provided in SEQ ID NO: 3 and SEQ ID NO: 4, respectively. The CXC motif corresponds to amino acid residues numbered 10-12. A predicted signal sequence is indicated, but may actually be of longer or shorter length depending, in part, upon the cell expressing the protein. The ELR motif immediately preceeding the CXC motif suggests function in inflammatory mediator trafficking.

The nucleic acid and amino acid sequences of the novel mouse 6Ckine (m6Ckine) chemokine are provided in SEQ ID NO: 5 and SEQ ID NO: 6, respectively. The predicted coding region begins at about position 71 and ends at position 469. A predicted signal sequence is indicated and the CC motif is at residues numbered 7-8. m6Ckine has a total of 6 conserved cysteine residues in the mature peptide, suggesting a new subclass of CC chemokines exhibiting extended carboxy terminal sequence.

The nucleic acid sequence and the corresponding predicted amino acid sequence of the novel human 6Ckine (h6Ckine) are provided in SEQ ID NO:7 and SEQ ID NO: 8,

respectively. Predicted coding sequence runs from position 6 through position 407. The CC motif corresponds to residues numbered 8-9; predicted signal sequence is indicated. h6Ckine has 6 conserved cysteine residues in the mature peptide, similar to m6Ckine, and exhibits carboxy terminal extension.

Table 1, below, depicts the alignment of porcine 6Ckine (p6Ckine) (SEQ ID NO: 9), mouse and human 6Ckine amino acid sequences.

Table 1: Alignment of porcine (SEQ ID NO: 9), mouse, and human 6Ckine amino acid sequences. "*" indicates identity, and "." denotes similarity. Only a portion of porcine 6Ckine is compared here. Other chemokines possess a structural domain which corresponds to the sequence from about Glu56 to Asp67. Immediately following this in the 6Ckines, both mouse and human, is additional sequence unlike other chemokines. Starting after Lys69 of human, are a series of prolines, which should disrupt helical structure. Thus the additional segment which contains two cysteines (at positions 80 and 99) appear peculiar to these molecules. These new structural features may define new biology and an additional chemokine subclass.

```
p6Ckine         MXQSLVLRILVLVLAFCIPHTQGSDGGAQDCCLKYSLRKIPTHVVRSYRK
m6Ckine         MAQMMTLSLLSLVLALCIPWTQGSDGGGQDCCLKYSQKKIPYSIVRGYRK
h6Ckine         MAQSLALSLLILVLAFGIPRTQGSDGGAQDCCLKYSQRKIPAKVVRSYRK
                * * . * .* ****  ** ******* ******** .***  .** ***

p6Ckine         QEPSLGCPIPAILFSPRKXSQPELCAD----------------------
m6Ckine         QEPSLGCPIPAILFSPRKHSKPELCANPEEGWVQNLMRRLDQPPAPGKQS
h6Ckine         QEPSLGCSIPAILFLPRKRSQAELCADPKELWVQQLMQHLDKTPSPQKPA
                ******* ****** *** *. ****** * *** **  **  * * *

p6Ckine         ----------------------------------
m6Ckine         PGCRKNRGTSKSGKKGKGSKGCKRTEQTQPSRG-
h6Ckine         QGCRKDRGASKTGKKGKGSKGCKRTERSQTPKGP
                **** ** ** **************  *  .*
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Partial amino acid sequence of human Chr19kine (CXC chemokine; SEQ ID NO: 10). The CXC motif is underlined. The absense of the ELR motif suggests anti-inflammatory properties. Chr19kine was found by search and careful analysis of a public EST database. See, e.g., gn1|dbest|14024 (H14024). This gene maps to human chromosome 19p12-13.1.

The chemokine proteins of this invention are defined in part by their physicochemical and biological properties. The biological properties of the chemokines described herein, e.g., mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine, are defined, in part, by their amino acid sequence, and mature size. They also should share at

least some biological properties with other similar chemokines. One of skill will readily recognize that some sequence variations may be tolerated, e.g., conservative substitutions or positions remote from the critical residues for receptor interaction or important tertiary structure features, without altering significantly the biological activity of the molecule. Conversely, non-conservative substitutions may be adapted to delete selected functions.

These chemokines are present in specific tissue types, e.g., lymphoid tissues, and the interaction of the protein with a receptor will be important for mediating various aspects of cellular physiology or development. The cellular types which express message encoding mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine suggest that signals important in cell differentiation and development are mediated by them. See, e.g., Gilbert (1991) Developmental Biology (3d ed.) Sinauer Associates, Sunderland, MA; Browder, et al. (1991) Developmental Biology (3d ed.) Saunders, Philadelphia, PA.; Russo, et al. (1992) Development: The Molecular Genetic Approach Springer-Verlag, New York, N.Y.; and Wilkins (1993) Genetic Analysis of Animal Development (2d ed.) Wiley-Liss, New York, N.Y. Moreover, mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine expression or responsiveness should serve as markers, e.g., to define certain cell subpopulations.

The mpf4 and mCTAP3 chemokines were discovered through searches and careful analysis of the GENBANK EST (WashU-Merck EST project, St. Louis, MO) public database. mpf4 exhibits approximately 71% amino acid identity to rat platelet factor 4 (pf4), which functions as a marker for megakaryocyte differentiation. See, e.g., Doi, et al. (1987) Mol. Cell. Biol. 7:898-904; and GenBank Accession number M15254 (1987). Identity comparisons of mCTAP3 sequence, see, e.g., Marra, et al. (1996) GenBank Accession number W53807, revealed 65% identity on the nucleotide level to human neutrophil-stimulating peptide 2 (NAP-2), and 55% identity on the amino acid level to

human connective tissue activating peptide 3 (hCTAP3). mCTAP3 is likely to be the murine counterpart of the human protein.

m6Ckine was also found by search and careful analysis of the public EST database with sequences exhibiting chemokine structure in the SWISS PROT database. The nucleotide sequence of Table 3 and SEQ ID NO 5 is found on three murine ESTs, see, e.g., gnl|dbest|17930 (W17930); gnl|dbest|67046 (W67046); and gnl|dbest|08383 (W08383). h6Ckine was found in the similar manner. The nucleotide sequence of Table 4 and SEQ ID NO: 6 can be found, e.g., on gnl|dbest|366929; gnl|dbest|302355; gnl|dbest|342967; gnl|dbest|356690; and on a clone designated est703.

Chr19kine was found by search and careful analysis of a public EST database. See, e.g., gnl|dbest|14024 (H14024). This gene maps to human chromosome 19p12-13.1.

Northern blot analysis was performed for m6Ckine using standard methods, see, e.g., Maniatis, et al. (1982) Molecular Cloning, A Laboratory Manual Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY; Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual (2d ed.) Vols. 1-3, CSH Press, NY; Ausubel, et al., Biology Greene Publishing Associates, Brooklyn, NY; and Ausubel, et al. (1987 and Supplements) Current Protocols in Molecular Biology Wiley/Greene, NY. Preliminary data indicates a transcript of approximately 900 bp with high levels of expression in mouse lung and spleen. Lower expression levels were found in mouse heart, liver, skeletal muscle, kidney, testis, and RAG 1 thymus tissue. No message has been detected in human fetal brain, lung, liver, or kidney tissues, nor has any expression been detected in various T cell libraries. However, analysis of human immune tissues yields expression in lymph node, appendix, and at low level, in spleen. This wide range of distribution in various tissues may indicate a role in normal leukocyte trafficking. Northern analysis of h6Ckine indicates expression in human tonsil, lymph node, and fetal spleen

tissues, as well germinal center cells. A lower message was found in fetal testis and small intestine.

Through standard Ca^{++} flux analysis, see, e.g., Coligan, et al (eds.)(1992 and periodic supplements) Current Protocols in Immunology, Greene/Wiley, NY, it was determined that the receptor for m6Ckine is CXCR3. This receptor is shared by other known chemokines, e.g., IP-10 and Mig. See, e.g., Sgadari, et al. (1997) Blood 89:2635-2643; and Loetscher, et al. (1996) J. Exp. Med. 184:963-969. This is an interesting ligand-receptor pair because as described above m6Ckine is a CC chemokine while CXCR3 normally binds CXC chemokines.

IP-10 and Mig are known to have angiostatic properties. See, e.g., Keane, et al. (1997) J. Immunol. 159:1437-1443; and Farber (1997) J. Leukoc. Biol. 61:246-257. Because of this angiostatic effect, Mig and IP-10 prevent tumor related angiogenesis as well as regulate the rate of wound healing and scar tissue formation. Since 6Ckine shares a receptor with IP-10 and Mig, 6Ckine may also play a role in preventing tumor formation, tumor metastasis, and/or regulating wound healing. Additionally, since 6Ckine is a T cell chemoattractant, especially for activated T cells, it may serve to enhance many T-cell mediated anti-tumor effects. This may occur with 6Ckine alone, or in combination with IP-10, Mig, lymphotactin, see, e.g., Kelner, et al. (1994) Science 266:1395-1399, and/or MIP3α, see, e.g., U.S.S.N. 08/675,814. And since h6Ckine binds to mouse CXCR3, it is likely that the chemokine will also bind to human CXCR3.

In addition, as for other ligands for the CXCR3 receptor, 6Ckine may mediate rapid lymphocyte adhesion. This may be useful in enhancing anti-tumor responses. Or, 6Ckine may be used to direct activated T cells to an antigen, e.g., an infectious agent.

II. Definitions

The term "binding composition" refers to molecules that bind with specificity and selectivity to a mpf4,

mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine, respectively, e.g., in an antibody-antigen interaction. However, other compounds, e.g., receptor proteins, may also specifically and/or selectively associate with mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokines to the exclusion of other molecules. Typically, the association will be in a natural physiologically relevant protein-protein interaction, either covalent or non-covalent, and may include members of a multiprotein complex, including carrier compounds or dimerization partners. The molecule may be a polymer, or chemical reagent. No implication as to whether a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine is either the ligand or the receptor of a ligand-receptor interaction is necessarily represented, other than whether the interaction exhibits similar specificity, e.g., specific affinity. A functional analog may be a ligand with structural modifications, or may be a wholly unrelated molecule, e.g., which has a molecular shape which interacts with the appropriate ligand binding determinants. The ligands may serve as agonists or antagonists of the receptor, see, e.g., Goodman, et al. (eds.) (1990) Goodman & Gilman's: The Pharmacological Bases of Therapeutics (8th ed.) Pergamon Press, Tarrytown, N.Y.

The term "binding agent:mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein complex", as used herein, refers to a complex of a binding agent and a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein that is formed by specific binding of the binding agent to the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein. Specific binding of the binding agent means that the binding agent has a specific binding site, e.g., antigen binding site, that recognizes a site on the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein. For example, antibodies raised to a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein and recognizing an epitope on the mpf4, CTAP3, or 6Ckine chemokine protein are capable of forming a binding agent:mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine

chemokine protein complex by specific binding. Typically, the formation of a binding agent:mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein complex allows the measurement of mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein in a mixture of other proteins and biologics. The term "antibody:mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein complex" refers to an embodiment in which the binding agent, e.g., is the antigen binding portion from an antibody. The antibody may be monoclonal, polyclonal, or a binding fragment of an antibody, e.g., an Fab or F(ab)2 fragment. The antibody will preferably be a polyclonal antibody for cross-reactivity testing purposes.

"Homologous" nucleic acid sequences, when compared, exhibit significant similarity, or identity. The standards for homology in nucleic acids are either measures for homology generally used in the art by sequence comparison and/or phylogenetic relationship, or based upon hybridization conditions. Hybridization conditions are described in greater detail below.

An "isolated" nucleic acid is a nucleic acid, e.g., an RNA, DNA, or a mixed polymer, which is substantially separated from other biologic components which naturally accompany a native sequence, e.g., proteins and flanking genomic sequences from the originating species. The term embraces a nucleic acid sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogs, or analogs biologically synthesized by heterologous systems. A substantially pure molecule includes isolated forms of the molecule. An isolated nucleic acid will usually contain homogeneous nucleic acid molecules, but will, in some embodiments, contain nucleic acids with minor sequence heterogeneity. This heterogeneity is typically found at the polymer ends or portions not critical to a desired biological function or activity.

As used herein, the term "mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein" shall encompass,

when used in a protein context, a protein having amino acid sequences, particularly from the chemokine motif portions, shown in SEQ ID NO: 2, 4, 6, 8, or 10, or a significant fragment unique to and/or characteristic of such a protein, preferably a natural embodiment. The invention also embraces a polypeptide which exhibits similar structure to mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine, e.g., which interacts with mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine specific binding components. These binding components, e.g., antibodies, typically bind to a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine with high affinity, e.g., at least about 100 nM, usually better than about 30 nM, preferably better than about 10 nM, and more preferably at better than about 3 nM.

The term "polypeptide" or "protein" as used herein includes a significant fragment or segment of chemokine motif portion of a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine, and encompasses a stretch of amino acid residues of at least about 8 amino acids, generally at least 10 amino acids, more generally at least 12 amino acids, often at least 14 amino acids, more often at least 16 amino acids, typically at least 18 amino acids, more typically at least 20 amino acids, usually at least 22 amino acids, more usually at least 24 amino acids, preferably at least 26 amino acids, more preferably at least 28 amino acids, and, in particularly preferred embodiments, at least about 30 or more amino acids, e.g., 35, 40, 45, 50, 60, 70, 80, etc. The segments may have amino and carboxy termini, with appropriate lengths, e.g., starting at residue 1, 2, 3, etc., and ending at residue 134, 133, 132, etc. The invention encompasses proteins comprising a plurality of said segments.

A "recombinant" nucleic acid is defined either by its method of production or its structure. In reference to its method of production, e.g., a product made by a process, the process is use of recombinant nucleic acid techniques, e.g., involving human intervention in the nucleotide sequence, typically selection or production.

Alternatively, it can be a nucleic acid made by generating a sequence comprising fusion of two fragments which are not naturally contiguous to each other, but is meant to exclude products of nature, e.g., naturally occurring mutants. Thus, for example, products made by transforming cells with any non-naturally occurring vector is encompassed, as are nucleic acids comprising sequence derived using any synthetic oligonucleotide process. Such is often done to replace a codon with a redundant codon encoding the same or a conservative amino acid, while typically introducing or removing a sequence recognition site. Alternatively, it is performed to join together nucleic acid segments of desired functions to generate a single genetic entity comprising a desired combination of functions not found in the commonly available natural forms. Restriction enzyme recognition sites are often the target of such artificial manipulations, but other site specific targets, e.g., promoters, DNA replication sites, regulation sequences, control sequences, or other useful features may be incorporated by design. A similar concept is intended for a recombinant, e.g., fusion, polypeptide. Specifically included are synthetic nucleic acids which, by genetic code redundancy, encode polypeptides similar to fragments of these antigens, and fusions of sequences from various different species variants.

"Solubility" is reflected by sedimentation measured in Svedberg units, which are a measure of the sedimentation velocity of a molecule under particular conditions. The determination of the sedimentation velocity was classically performed in an analytical ultracentrifuge, but is typically now performed in a standard ultracentrifuge. See, Freifelder (1982) Physical Biochemistry (2d ed.) W.H. Freeman & Co., San Francisco, CA; and Cantor and Schimmel (1980) Biophysical Chemistry parts 1-3, W.H. Freeman & Co., San Francisco, CA. As a crude determination, a sample containing a putatively soluble polypeptide is spun in a standard full sized ultracentrifuge at about 50K rpm for about 10

minutes, and soluble molecules will remain in the supernatant. A soluble particle or polypeptide will typically be less than about 30S, more typically less than about 15S, usually less than about 10S, more usually less than about 6S, and, in particular embodiments, preferably less than about 4S, and more preferably less than about 3S. Solubility of a polypeptide or fragment depends upon the environment and the polypeptide. Many parameters affect polypeptide solubility, including temperature, electrolyte environment, size and molecular characteristics of the polypeptide, and nature of the solvent. Typically, the temperature at which the polypeptide is used ranges from about 4° C to about 65° C. Usually the temperature at use is greater than about 18° C and more usually greater than about 22° C. For diagnostic purposes, the temperature will usually be about room temperature or warmer, but less than the denaturation temperature of components in the assay. For therapeutic purposes, the temperature will usually be body temperature, typically about 37° C for humans, though under certain situations the temperature may be raised or lowered in situ or in vitro.

The size and structure of the polypeptide should generally be in a substantially stable state, and usually not in a denatured state. The polypeptide may be associated with other polypeptides in a quaternary structure, e.g., to confer solubility, or associated with lipids or detergents in a manner which approximates natural lipid bilayer interactions.

The solvent will usually be a biologically compatible buffer, of a type used for preservation of biological activities, and will usually approximate a physiological solvent. Usually the solvent will have a neutral pH, typically between about 5 and 10, and preferably about 7.5. On some occasions, a detergent will be added, typically a mild non-denaturing one, e.g., CHS (cholesteryl hemisuccinate) or CHAPS (3-[3-cholamidopropyl- dimethylammonio]-1-propane sulfonate), or a low enough concentration as to avoid significant

disruption of structural or physiological properties of the protein.

"Substantially pure" in a protein context typically means that the protein is isolated from other contaminating proteins, nucleic acids, and other biologicals derived from the original source organism. Purity, or "isolation" may be assayed by standard methods, and will ordinarily be at least about 50% pure, more ordinarily at least about 60% pure, generally at least about 70% pure, more generally at least about 80% pure, often at least about 85% pure, more often at least about 90% pure, preferably at least about 95% pure, more preferably at least about 98% pure, and in most preferred embodiments, at least 99% pure. Similar concepts apply, e.g., to antibodies or nucleic acids.

"Substantial similarity" in the nucleic acid sequence comparison context means either that the segments, or their complementary strands, when compared, are identical when optimally aligned, with appropriate nucleotide insertions or deletions, in at least about 50% of the nucleotides, generally at least 56%, more generally at least 59%, ordinarily at least 62%, more ordinarily at least 65%, often at least 68%, more often at least 71%, typically at least 74%, more typically at least 77%, usually at least 80%, more usually at least about 85%, preferably at least about 90%, more preferably at least about 95 to 98% or more, and in particular embodiments, as high at about 99% or more of the nucleotides. Alternatively, substantial similarity exists when the segments will hybridize under selective hybridization conditions, to a strand, or its complement, typically using a sequence derived from SEQ ID NO: 1, 3, 5, or 7. Typically, selective hybridization will occur when there is at least about 55% similarity over a stretch of at least about 30 nucleotides, preferably at least about 65% over a stretch of at least about 25 nucleotides, more preferably at least about 75%, and most preferably at least about 90% over about 20 nucleotides. See Kanehisa (1984) Nuc. Acids Res. 12:203-213. The

length of similarity comparison, as described, may be over longer stretches, and in certain embodiments will be over a stretch of at least about 17 nucleotides, usually at least about 20 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 40 nucleotides, preferably at least about 50 nucleotides, and more preferably at least about 75 to 100 or more nucleotides, e.g., 150, 200, etc.

"Stringent conditions", in referring to homology or substantial similarity in the hybridization context, will be stringent combined conditions of salt, temperature, organic solvents, and other parameters, typically those controlled in hybridization reactions. The combination of parameters is more important than the measure of any single parameter. See, e.g., Wetmur and Davidson (1968) J. Mol. Biol. 31:349-370. A nucleic acid probe which binds to a target nucleic acid under stringent conditions is specific for said target nucleic acid. Such a probe is typically more than 11 nucleotides in length, and is sufficiently identical or complementary to a target nucleic acid over the region specified by the sequence of the probe to bind the target under stringent hybridization conditions.

mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokines from other mammalian species can be cloned and isolated by cross-species hybridization of closely related species. See, e.g., below. Similarity may be relatively low between distantly related species, and thus hybridization of relatively closely related species is advisable. Alternatively, preparation of an antibody preparation which exhibits less species specificity may be useful in expression cloning approaches.

The phrase "specifically binds to an antibody" or "specifically immunoreactive with", when referring to a protein or peptide, refers to a binding reaction which is determinative of the presence of the protein in the presence of a heterogeneous population of proteins and other biological components. Thus, under designated

immunoassay conditions, the specified antibodies bind to a particular protein and do not significantly bind other proteins present in the sample. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, antibodies raised to the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein immunogen with the amino acid sequence depicted in SEQ ID NO: 2, 4, 6, 8, or 10 can be selected to obtain antibodies specifically immunoreactive with mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine proteins and not with other proteins. The antibodies may be species specific, e.g., also recognizing polymorphic and splicing or developmental variants.

III. Nucleic Acids

mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine is exemplary of structurally and functionally related proteins. These soluble chemokine proteins will serve to transmit signals between different cell types. The preferred embodiments, as disclosed, will be useful in standard procedures to isolate genes from different individuals or other species, e.g., warm blooded animals, such as birds and mammals. Cross hybridization will allow isolation of related genes encoding proteins from individuals, strains, or species. A number of different approaches are available to successfully isolate a suitable nucleic acid clone based upon the information provided herein. Southern blot hybridization studies can qualitatively determine the presence of homologous genes in human, monkey, rat, mouse, dog, cat, cow, and rabbit genomes under specific hybridization conditions.

Complementary sequences will also be used as probes or primers. Based upon identification of the likely amino terminus, other peptides should be particularly useful, e.g., coupled with anchored vector or poly-A complementary PCR techniques or with complementary DNA of other peptides.

Techniques for nucleic acid manipulation of genes encoding mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine proteins, such as subcloning nucleic acid sequences encoding polypeptides into expression vectors, labeling probes, DNA hybridization, and the like are described generally in Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual (2nd ed.) Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY, which is incorporated herein by reference. This manual is hereinafter referred to as "Sambrook, et al."

There are various methods of isolating DNA sequences encoding mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine proteins. For example, DNA is isolated from a genomic or cDNA library using labeled oligonucleotide probes having sequences identical or complementary to the sequences disclosed herein. Full-length probes may be used, or oligonucleotide probes may be generated by comparison of the sequences disclosed. Such probes can be used directly in hybridization assays to isolate DNA encoding mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine proteins, or probes can be designed for use in amplification techniques such as PCR, for the isolation of DNA encoding mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine proteins. Reverse translation computer programs can also provide alternative nucleic acid sequences which encode the same proteins.

To prepare a cDNA library, mRNA is isolated from cells which expresses a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein. cDNA is prepared from the mRNA and ligated into a recombinant vector. The vector is transfected into a recombinant host for propagation, screening, and cloning. Methods for making and screening cDNA libraries are well known. See Gubler and Hoffman (1983) Gene 25:263-269 and Sambrook, et al.

For a genomic library, the DNA can be extracted from tissue and either mechanically sheared or enzymatically digested to yield fragments of about 12-20 kb. The fragments are then separated by gradient centrifugation and cloned in bacteriophage lambda vectors. These

vectors and phage are packaged in vitro, as described in Sambrook, et al. Recombinant phage are analyzed by plaque hybridization as described in Benton and Davis (1977) Science 196:180-182. Colony hybridization is carried out as generally described in e.g., Grunstein, et al. (1975) Proc. Natl. Acad. Sci. USA. 72:3961-3965.

DNA encoding a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein can be identified in either cDNA or genomic libraries by its ability to hybridize with the nucleic acid probes described herein, e.g., in colony or plaque hybridization assays. The corresponding DNA regions are isolated by standard methods familiar to those of skill in the art. See, e.g., Sambrook, et al.

Various methods of amplifying target sequences, such as the polymerase chain reaction, can also be used to prepare DNA encoding mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine proteins. Polymerase chain reaction (PCR) technology is used to amplify such nucleic acid sequences directly from mRNA, from cDNA, and from genomic libraries or cDNA libraries. The isolated sequences encoding mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine proteins may also be used as templates for PCR amplification.

Typically, in PCR techniques, oligonucleotide primers complementary to two 5' regions in the DNA region to be amplified are synthesized. The polymerase chain reaction is then carried out using the two primers. See Innis, et al. (eds.) (1990) PCR Protocols: A Guide to Methods and Applications Academic Press, San Diego, CA. Primers can be selected to amplify the entire regions encoding a full-length mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein or to amplify smaller DNA segments as desired. Once such regions are PCR-amplified, they can be sequenced and oligonucleotide probes can be prepared from sequence obtained using standard techniques. These probes can then be used to isolate DNA's encoding mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine proteins.

Oligonucleotides for use as probes are usually chemically synthesized according to the solid phase phosphoramidite triester method first described by Beaucage and Carruthers (1983) Tetrahedron Lett. 22(20):1859-1862, or using an automated synthesizer, as described in Needham-VanDevanter, et al. (1984) Nucleic Acids Res. 12:6159-6168. Purification of oligonucleotides is performed e.g., by native acrylamide gel electrophoresis or by anion-exchange HPLC as described in Pearson and Regnier (1983) J. Chrom. 255:137-149. The sequence of the synthetic oligonucleotide can be verified using, e.g., the chemical degradation method of Maxam, A.M. and Gilbert, W. in Grossman, L. and Moldave (eds.) (1980) Methods in Enzymology 65:499-560 Academic Press, New York.

An isolated nucleic acid encoding a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein was identified. The nucleotide sequence and corresponding open reading frame are provided in SEQ ID NO: 1, 3, 5, or 7.

These mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokines exhibit limited similarity to portions of chemokines. See, e.g., Matsushima and Oppenheim (1989) Cytokine 1:2-13; Oppenheim, et al. (1991) Ann. Rev. Immunol. 9:617-648; Schall (1991) Cytokine 3:165-183; and Gronenborn and Clore (1991) Protein Engineering 4:263-269. Other features of comparison are apparent between the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine and chemokine families. See, e.g., Lodi, et al. (1994) Science 263:1762-1766. In particular, β-sheet and α-helix residues can be determined using, e.g., RASMOL program, see Sayle and Milner-White (1995) TIBS 20:374-376; or Gronenberg, et al. (1991) Protein Engineering 4:263-269; and other structural features are defined in Lodi, et al. (1994) Science 263:1762-1767. These secondary and tertiary features assist in defining further the C, CC, CXC, and CX3C structural features, along with spacing of appropriate cysteine residues. The 6Ckine embodiments provided herein exhibit peculiar

carboxy termini for the chemokine class of molecules. In particular, the sequence of h6Ckine extending beyond residue Asp68 to Pro111 therein may be peculiar to, and may represent a heretofore unrecognized subgroup of the CC chemokines.

This invention provides isolated DNA or fragments to encode a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein. In addition, this invention provides isolated or recombinant DNA which encodes a protein or polypeptide which is capable of hybridizing under appropriate conditions, e.g., high stringency, with the DNA sequences described herein. Said biologically active protein or polypeptide can be an intact ligand, or fragment, and have an amino acid sequence as disclosed in SEQ ID NO: 2, 4, 6, 8, or 10, particularly natural embodiments. Preferred embodiments will be full length natural sequences, from isolates, e.g., about 11,000 to 12,500 daltons in size when unglycosylated, or fragments of at least about 6,000 daltons, more preferably at least about 8,000 daltons. In glycosylated form, the protein may exceed 12,500 daltons. The 6Ckine embodiments exhibit a correspondingly larger size. Further, this invention contemplates the use of isolated or recombinant DNA, or fragments thereof, which encode proteins which are homologous to a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein or which were isolated using cDNA encoding a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein as a probe. The isolated DNA can have the respective regulatory sequences in the 5' and 3' flanks, e.g., promoters, enhancers, poly-A addition signals, and others. Also embraced are methods for making expression vectors with these sequences, or for making, e.g., expressing and purifying, protein products.

IV. Making mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokines

DNAs which encode a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine or fragments thereof can be

obtained by chemical synthesis, screening cDNA libraries, or by screening genomic libraries prepared from a wide variety of cell lines or tissue samples. Methods for doing so, or making expression vectors are described herein.

These DNAs can be expressed in a wide variety of host cells for the synthesis of a full-length protein or fragments which can in turn, e.g., be used to generate polyclonal or monoclonal antibodies; for binding studies; for construction and expression of modified molecules; and for structure/function studies. Each mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine or its fragments can be expressed in host cells that are transformed or transfected with appropriate expression vectors. These molecules can be substantially purified to be free of protein or cellular contaminants, other than those derived from the recombinant host, and therefore are particularly useful in pharmaceutical compositions when combined with a pharmaceutically acceptable carrier and/or diluent. The antigen, e.g., mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine, or portions thereof, may be expressed as fusions with other proteins or possessing an epitope tag.

Expression vectors are typically self-replicating DNA or RNA constructs containing the desired antigen gene or its fragments, usually operably linked to appropriate genetic control elements that are recognized in a suitable host cell. The specific type of control elements necessary to effect expression will depend upon the eventual host cell used. Generally, the genetic control elements can include a prokaryotic promoter system or a eukaryotic promoter expression control system, and typically include a transcriptional promoter, an optional operator to control the onset of transcription, transcription enhancers to elevate the level of mRNA expression, a sequence that encodes a suitable ribosome binding site, and sequences that terminate transcription and translation. Expression vectors also usually contain an origin of replication

that allows the vector to replicate independently from the host cell.

The vectors of this invention contain DNAs which encode a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine, or a fragment thereof, typically encoding, e.g., a biologically active polypeptide, or protein. The DNA can be under the control of a viral promoter and can encode a selection marker. This invention further contemplates use of such expression vectors which are capable of expressing eukaryotic cDNA coding for a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein in a prokaryotic or eukaryotic host, where the vector is compatible with the host and where the eukaryotic cDNA coding for the protein is inserted into the vector such that growth of the host containing the vector expresses the cDNA in question. Usually, expression vectors are designed for stable replication in their host cells or for amplification to greatly increase the total number of copies of the desirable gene per cell. It is not always necessary to require that an expression vector replicate in a host cell, e.g., it is possible to effect transient expression of the protein or its fragments in various hosts using vectors that do not contain a replication origin that is recognized by the host cell. It is also possible to use vectors that cause integration of a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine gene or its fragments into the host DNA by recombination, or to integrate a promoter which controls expression of an endogenous gene.

Vectors, as used herein, contemplate plasmids, viruses, bacteriophage, integratable DNA fragments, and other vehicles which enable the integration of DNA fragments into the genome of the host. Expression vectors are specialized vectors which contain genetic control elements that effect expression of operably linked genes. Plasmids are the most commonly used form of vector, but many other forms of vectors which serve an equivalent function are suitable for use herein. See, e.g., Pouwels, et al. (1985 and Supplements) Cloning

Vectors: A Laboratory Manual Elsevier, N.Y.; and Rodriquez, et al. (eds.) (1988) Vectors: A Survey of Molecular Cloning Vectors and Their Uses Buttersworth, Boston, MA.

Suitable host cells include prokaryotes, lower eukaryotes, and higher eukaryotes. Prokaryotes include both gram negative and gram positive organisms, e.g., E. coli and B. subtilis. Lower eukaryotes include yeasts, e.g., S. cerevisiae and Pichia, and species of the genus Dictyostelium. Higher eukaryotes include established tissue culture cell lines from animal cells, both of non-mammalian origin, e.g., insect cells, and birds, and of mammalian origin, e.g., human, primates, and rodents.

Prokaryotic host-vector systems include a wide variety of vectors for many different species. As used herein, E. coli and its vectors will be used generically to include equivalent vectors used in other prokaryotes. A representative vector for amplifying DNA is pBR322 or its derivatives. Vectors that can be used to express mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokines or mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine fragments include, but are not limited to, such vectors as those containing the lac promoter (pUC-series); trp promoter (pBR322-trp); Ipp promoter (the pIN-series); lambda-pP or pR promoters (pOTS); or hybrid promoters such as ptac (pDR540). See Brosius, et al. (1988) "Expression Vectors Employing Lambda-, trp-, lac-, and Ipp-derived Promoters", in Rodriguez and Denhardt (eds.) Vectors: A Survey of Molecular Cloning Vectors and Their Uses 10:205-236 Buttersworth, Boston, MA.

Lower eukaryotes, e.g., yeasts and Dictyostelium, may be transformed with mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine sequence containing vectors. For purposes of this invention, the most common lower eukaryotic host is the baker's yeast, Saccharomyces cerevisiae. It will be used generically to represent lower eukaryotes although a number of other strains and species are also available. Yeast vectors typically consist of a replication origin (unless of the

integrating type), a selection gene, a promoter, DNA encoding the desired protein or its fragments, and sequences for translation termination, polyadenylation, and transcription termination. Suitable expression vectors for yeast include such constitutive promoters as 3-phosphoglycerate kinase and various other glycolytic enzyme gene promoters or such inducible promoters as the alcohol dehydrogenase 2 promoter or metallothionine promoter. Suitable vectors include derivatives of the following types: self-replicating low copy number (such as the YRp-series), self-replicating high copy number (such as the YEp-series); integrating types (such as the YIp-series), or mini-chromosomes (such as the YCp-series).

Higher eukaryotic tissue culture cells are typically the preferred host cells for expression of the functionally active mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein. In principle, many higher eukaryotic tissue culture cell lines may be used, e.g., insect baculovirus expression systems, whether from an invertebrate or vertebrate source. However, mammalian cells are preferred to achieve proper processing, both cotranslationally and posttranslationally.

Transformation or transfection and propagation of such cells is routine. Useful cell lines include HeLa cells, Chinese hamster ovary (CHO) cell lines, baby rat kidney (BRK) cell lines, insect cell lines, bird cell lines, and monkey (COS) cell lines. Expression vectors for such cell lines usually include an origin of replication, a promoter, a translation initiation site, RNA splice sites (e.g., if genomic DNA is used), a polyadenylation site, and a transcription termination site. These vectors also may contain a selection gene or amplification gene. Suitable expression vectors may be plasmids, viruses, or retroviruses carrying promoters derived, e.g., from such sources as from adenovirus, SV40, parvoviruses, vaccinia virus, or cytomegalovirus. Representative examples of suitable expression vectors include pCDNA1; pCD, see Okayama, et al. (1985) Mol. Cell Biol. 5:1136-1142;

pMC1neo Poly-A, see Thomas, et al. (1987) Cell 51:503-512; and a baculovirus vector such as pAC 373 or pAC 610.

It is likely that mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokines need not be glycosylated to elicit biological responses. However, it will occasionally be desirable to express a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine polypeptide in a system which provides a specific or defined glycosylation pattern. In this case, the usual pattern will be that provided naturally by the expression system. However, the pattern will be modifiable by exposing the polypeptide, e.g., in unglycosylated form, to appropriate glycosylating proteins introduced into a heterologous expression system. For example, the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine gene may be co-transformed with one or more genes encoding mammalian or other glycosylating enzymes. It is further understood that over glycosylation may be detrimental to mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine biological activity, and that one of skill may perform routine testing to optimize the degree of glycosylation which confers optimal biological activity.

A mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine, or a fragment thereof, may be engineered to be phosphatidyl inositol (PI) linked to a cell membrane, but can be removed from membranes by treatment with a phosphatidyl inositol cleaving enzyme, e.g., phosphatidyl inositol phospholipase-C. This releases the antigen in a biologically active form, and allows purification by standard procedures of protein chemistry. See, e.g., Low (1989) Biochem. Biophys. Acta 988:427-454; Tse, et al. (1985) Science 230:1003-1008; and Brunner, et al. (1991) J. Cell Biol. 114:1275-1283.

Now that mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokines have been characterized, fragments or derivatives thereof can be prepared by conventional processes for synthesizing peptides. These include processes such as are described in Stewart and Young (1984) Solid Phase Peptide Synthesis Pierce Chemical Co.,

Rockford, IL; Bodanszky and Bodanszky (1984) The Practice of Peptide Synthesis Springer-Verlag, New York, NY; and Bodanszky (1984) The Principles of Peptide Synthesis Springer-Verlag, New York, NY. For example, an azide process, an acid chloride process, an acid anhydride process, a mixed anhydride process, an active ester process (for example, p-nitrophenyl ester, N-hydroxysuccinimide ester, or cyanomethyl ester), a carbodiimidazole process, an oxidative-reductive process, or a dicyclohexylcarbodiimide (DCCD)/additive process can be used. Solid phase and solution phase syntheses are both applicable to the foregoing processes. See also chemical ligation, e.g., Dawson, et al. (1994) Science 266:776-779, a method of linking long synthetic peptides by a peptide bond.

The prepared protein and fragments thereof can be isolated and purified from the reaction mixture by means of peptide separation, for example, by extraction, precipitation, electrophoresis and various forms of chromatography, and the like. The mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokines of this invention can be obtained in varying degrees of purity depending upon its desired use. Purification can be accomplished by use of known protein purification techniques or by the use of the antibodies or binding partners herein described, e.g., in immunoabsorbant affinity chromatography. This immunoabsorbant affinity chromatography is carried out by first linking the antibodies to a solid support and then contacting the linked antibodies with solubilized lysates of appropriate source cells, lysates of other cells expressing the ligand, or lysates or supernatants of cells producing the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokines as a result of recombinant DNA techniques, see below.

Multiple cell lines may be screened for one which expresses a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine at a high level compared with other cells. Natural mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokines can be isolated from natural sources, or by

expression from a transformed cell using an appropriate expression vector. Purification of the expressed protein is achieved by standard procedures, or may be combined with engineered means for effective purification at high efficiency from cell lysates or supernatants. Epitope or other tags, e.g., FLAG or His_6 segments, can be used for such purification features.

V. Antibodies

Antibodies can be raised to various mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokines, including individual, polymorphic, allelic, strain, or species variants, and fragments thereof, both in their naturally occurring (full-length) forms and in their recombinant forms. Additionally, antibodies can be raised to mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokines in either their active forms or in their inactive forms. Anti-idiotypic antibodies may also be used. The antibodies may exhibit various binding specificities for species, individual or polymorphic variants

A. Antibody Production

A number of immunogens may be used to produce antibodies specifically reactive with mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine proteins. Recombinant protein is the preferred immunogen for the production of monoclonal or polyclonal antibodies. Naturally occurring protein may also be used either in pure or impure form. Synthetic peptides, made using the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein sequences described herein, may also used as an immunogen for the production of antibodies to mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokines. Recombinant protein can be expressed in eukaryotic or prokaryotic cells as described herein, and purified as described. Naturally folded or denatured material can be used, as appropriate, for producing antibodies. Either monoclonal or polyclonal antibodies may be generated for subsequent use in immunoassays to measure the protein.

Methods of producing polyclonal antibodies are known to those of skill in the art. Typically, an immunogen, preferably a purified protein, is mixed with an adjuvant and animals are immunized with the mixture. The animal's immune response to the immunogen preparation is monitored by taking test bleeds and determining the titer of reactivity to the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein of interest. When appropriately high titers of antibody to the immunogen are obtained, usually after repeated immunizations, blood is collected from the animal and antisera are prepared. Further fractionation of the antisera to enrich for antibodies reactive to the protein can be done if desired. See, e.g., Harlow and Lane; or Coligan.

Monoclonal antibodies may be obtained by various techniques familiar to those skilled in the art. Typically, spleen cells from an animal immunized with a desired antigen are immortalized, commonly by fusion with a myeloma cell (see, Kohler and Milstein (1976) Eur. J. Immunol. 6:511-519, incorporated herein by reference). Alternative methods of immortalization include transformation with Epstein Barr Virus, oncogenes, or retroviruses, or other methods known in the art. Colonies arising from single immortalized cells are screened for production of antibodies of the desired specificity and affinity for the antigen, and yield of the monoclonal antibodies produced by such cells may be enhanced by various techniques, including injection into the peritoneal cavity of a vertebrate host. Alternatively, one may isolate DNA sequences which encode a monoclonal antibody or a binding fragment thereof by screening a DNA library from human B cells according, e.g., to the general protocol outlined by Huse, et al. (1989) Science 246:1275-1281.

Antibodies, including binding fragments and single chain versions, against predetermined fragments of mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokines can be raised by immunization of animals with conjugates of the fragments with carrier proteins as described above.

Monoclonal antibodies are prepared from cells secreting the desired antibody. These antibodies can be screened for binding to normal or defective mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokines, or screened for agonistic or antagonistic activity, e.g., mediated through a receptor. These monoclonal antibodies will usually bind with at least a K_D of about 1 mM, more usually at least about 300 μM, typically at least about 10 μM, more typically at least about 30 μM, preferably at least about 10 μM, and more preferably at least about 3 μM or better.

In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts, such as mice, rodents, primates, humans, etc. Description of techniques for preparing such monoclonal antibodies may be found in, e.g., Stites, et al. (eds.) Basic and Clinical Immunology (4th ed.) Lange Medical Publications, Los Altos, CA, and references cited therein; Harlow and Lane (1988) Antibodies: A Laboratory Manual CSH Press; Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed.) Academic Press, New York, NY; and particularly in Kohler and Milstein (1975) Nature 256:495-497, which discusses one method of generating monoclonal antibodies. Summarized briefly, this method involves injecting an animal with an immunogen. The animal is then sacrificed and cells taken from its spleen, which are then fused with myeloma cells. The result is a hybrid cell or "hybridoma" that is capable of reproducing in vitro. The population of hybridomas is then screened to isolate individual clones, each of which secrete a single antibody species to the immunogen. In this manner, the individual antibody species obtained are the products of immortalized and cloned single B cells from the immune animal generated in response to a specific site recognized on the immunogenic substance.

Other suitable techniques involve selection of libraries of antibodies in phage or similar vectors. See, e.g., Huse, et al. (1989) "Generation of a Large Combinatorial Library of the Immunoglobulin Repertoire in

Phage Lambda," Science 246:1275-1281; and Ward, et al. (1989) Nature 341:544-546. The polypeptides and antibodies of the present invention may be used with or without modification, including chimeric or humanized antibodies. Frequently, the polypeptides and antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like. Patents, teaching the use of such labels include U.S. Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant immunoglobulins may be produced. See, Cabilly, U.S. Patent No. 4,816,567; and Queen, et al. (1989) Proc. Nat'l Acad. Sci. USA 86:10029-10033.

The antibodies of this invention are useful for affinity chromatography in isolating mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein. Columns can be prepared where the antibodies are linked to a solid support, e.g., particles, such as agarose, SEPHADEX, or the like, where a cell lysate or supernatant may be passed through the column, the column washed, followed by increasing concentrations of a mild denaturant, whereby purified mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein will be released. Likewise, antibody binding to the chemokine may be capable of neutralizing receptor binding, and may serve as a receptor antagonist. They may also be useful as Western blot detection reagents, or ELISA reagents.

The antibodies may also be used to screen expression libraries for particular expression products. Usually the antibodies used in such a procedure will be labeled with a moiety allowing easy detection of presence of antigen by antibody binding.

Antibodies to mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokines may be used for the identification of cell populations expressing mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokines. By assaying the expression products of cells expressing mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokines it is possible to diagnose disease, e.g., immune-compromised conditions.

Antibodies raised against each mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine will also be useful to raise anti-idiotypic antibodies. These will be useful in detecting or diagnosing various immunological conditions related to expression of the respective antigens.

B. Immunoassays

A particular protein can be measured by a variety of immunoassay methods. For a review of immunological and immunoassay procedures in general, see Stites and Terr (eds.) (1991) Basic and Clinical Immunology (7th ed.). Moreover, the immunoassays of the present invention can be performed in many configurations, which are reviewed extensively in Maggio (ed.) (1980) Enzyme Immunoassay CRC Press, Boca Raton, Florida; Tijan (1985) "Practice and Theory of Enzyme Immunoassays," Laboratory Techniques in Biochemistry and Molecular Biology, Elsevier Science Publishers B.V., Amsterdam; and Harlow and Lane Antibodies, A Laboratory Manual, supra, each of which is incorporated herein by reference. See also Chan (ed.) (1987) Immunoassay: A Practical Guide Academic Press, Orlando, FL; Price and Newman (eds.) (1991) Principles and Practice of Immunoassays Stockton Press, NY; and Ngo (ed.) (1988) Non-isotopic Immunoassays Plenum Press, NY.

Immunoassays for measurement of mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine proteins can be performed by a variety of methods known to those skilled in the art. In brief, immunoassays to measure the protein can be either competitive or noncompetitive binding assays. In competitive binding assays, the sample to be analyzed competes with a labeled analyte for specific binding sites on a capture agent bound to a

solid surface. Preferably the capture agent is an antibody specifically reactive with mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine proteins produced as described above. The concentration of labeled analyte bound to the capture agent is inversely proportional to the amount of free analyte present in the sample.

In a competitive binding immunoassay, the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein present in the sample competes with labeled protein for binding to a specific binding agent, for example, an antibody specifically reactive with the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein. The binding agent may be bound to a solid surface to effect separation of bound labeled protein from the unbound labeled protein. Alternately, the competitive binding assay may be conducted in liquid phase and a variety of techniques known in the art may be used to separate the bound labeled protein from the unbound labeled protein. Following separation, the amount of bound labeled protein is determined. The amount of protein present in the sample is inversely proportional to the amount of labeled protein binding.

Alternatively, a homogeneous immunoassay may be performed in which a separation step is not needed. In these immunoassays, the label on the protein is altered by the binding of the protein to its specific binding agent. This alteration in the labeled protein results in a decrease or increase in the signal emitted by label, so that measurement of the label at the end of the immunoassay allows for detection or quantitation of the protein.

mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine proteins may also be determined by a variety of noncompetitive immunoassay methods. For example, a two-site, solid phase sandwich immunoassay may be used. In this type of assay, a binding agent for the protein, for example an antibody, is attached to a solid support. A second protein binding agent, which may also be an

antibody, and which binds the protein at a different site, is labeled. After binding at both sites on the protein has occurred, the unbound labeled binding agent is removed and the amount of labeled binding agent bound to the solid phase is measured. The amount of labeled binding agent bound is directly proportional to the amount of protein in the sample.

Western blot analysis can be used to determine the presence of mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine proteins in a sample. Electrophoresis is carried out, for example, on a tissue sample suspected of containing the protein. Following electrophoresis to separate the proteins, and transfer of the proteins to a suitable solid support, e.g., a nitrocellulose filter, the solid support is incubated with an antibody reactive with the protein. This antibody may be labeled, or alternatively may be detected by subsequent incubation with a second labeled antibody that binds the primary antibody.

The immunoassay formats described above employ labeled assay components. The label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art. A wide variety of labels and methods may be used. Traditionally, a radioactive label incorporating ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P was used. Non-radioactive labels include ligands which bind to labeled antibodies, fluorophores, chemiluminescent agents, enzymes, and antibodies which can serve as specific binding pair members for a labeled ligand. The choice of label depends on sensitivity required, ease of conjugation with the compound, stability requirements, and available instrumentation. For a review of various labeling or signal producing systems which may be used, see U.S. Patent No. 4,391,904, which is incorporated herein by reference.

Antibodies reactive with a particular protein can also be measured by a variety of immunoassay methods. For a review of immunological and immunoassay procedures

applicable to the measurement of antibodies by immunoassay techniques, see Stites and Terr (eds.) Basic and Clinical Immunology (7th ed.) supra; Maggio (ed.) Enzyme Immunoassay, supra; and Harlow and Lane Antibodies, A Laboratory Manual, supra.

In brief, immunoassays to measure antisera reactive with mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine proteins can be either competitive or noncompetitive binding assays. In competitive binding assays, the sample analyte competes with a labeled analyte for specific binding sites on a capture agent bound to a solid surface. Preferably the capture agent is a purified recombinant mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein produced as described above. Other sources of mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine proteins, including isolated or partially purified naturally occurring protein, may also be used. Noncompetitive assays include sandwich assays, in which the sample analyte is bound between two analyte-specific binding reagents. One of the binding agents is used as a capture agent and is bound to a solid surface. The second binding agent is labeled and is used to measure or detect the resultant complex by visual or instrument means. A number of combinations of capture agent and labeled binding agent can be used. A variety of different immunoassay formats, separation techniques, and labels can be also be used similar to those described above for the measurement of mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine proteins.

VI. Purified mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokines

Mouse nucleotide and amino acid sequences are provided in SEQ ID NO: 1 to 6, and human nucleotide and amino acid sequences for h6Ckine are provided in SEQ ID NO: 7 and 8.

Purified protein or defined peptides are useful for generating antibodies by standard methods, as described above. Synthetic peptides or purified protein can be

presented to an immune system to generate polyclonal and monoclonal antibodies. See, e.g., Coligan (1991) Current Protocols in Immunology Wiley/Greene, NY; and Harlow and Lane (1989) Antibodies: A Laboratory Manual Cold Spring Harbor Press, NY, which are incorporated herein by reference. Alternatively, a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine receptor can be useful as a specific binding reagent, and advantage can be taken of its specificity of binding, for, e.g., purification of a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine ligand.

The specific binding composition can be used for screening an expression library made from a cell line which expresses a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine. Many methods for screening are available, e.g., standard staining of surface expressed ligand, or by panning. Screening of intracellular expression can also be performed by various staining or immunofluorescence procedures. The binding compositions could be used to affinity purify or sort out cells expressing the ligand.

The peptide segments, along with comparison to homologous genes, can also be used to produce appropriate oligonucleotides to screen a library. The genetic code can be used to select appropriate oligonucleotides useful as probes for screening. In combination with polymerase chain reaction (PCR) techniques, synthetic oligonucleotides will be useful in selecting desired clones from a library, including natural allelic an polymorphic variants.

The peptide sequences allow preparation of peptides to generate antibodies to recognize such segments, and allow preparation of oligonucleotides which encode such sequences. The sequence also allows for synthetic preparation, e.g., see Dawson, et al. (1994) Science 266:776-779. Since mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokines may be secreted proteins, the gene will normally possess an N-terminal signal sequence, which is removed upon processing and secretion. However,

the exact processing point may be vary in different cell types, and forms of different lengths are often detected. Prediction of the signal cleavage point can be performed, e.g., using the methods of Nielsen, et al. (1997) Protein Eng. 10:1-8. Analysis of the structural features in comparison with the most closely related reported sequences has revealed similarities with other cytokines, particularly the class of proteins known as CC and CXC chemokines. The longer carboxy terminus of the 6Ckine embodiments suggest a distinct subfamily of CC chemokines. This subfamily may exhibit additional biological or structural features.

VII. Physical Variants

This invention also encompasses proteins or peptides having substantial amino acid sequence similarity with an amino acid sequence of a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine. Natural variants include individual, polymorphic, allelic, strain, or species variants.

Amino acid sequence similarity, or sequence identity, is determined by optimizing residue matches, if necessary, by introducing gaps as required. This changes when considering conservative substitutions as matches. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. Homologous amino acid sequences include natural polymorphic, allelic, and interspecies variations in each respective protein sequence. Typical homologous proteins or peptides will have from 50-100% similarity (if gaps can be introduced), to 75-100% similarity (if conservative substitutions are included) with the amino acid sequence of the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine. Similarity measures will be at least about 50%, generally at least 60%, more generally at least 65%, usually at least 70%, more usually at least

75%, preferably at least 80%, and more preferably at least 80%, and in particularly preferred embodiments, at least 85% or more. See also Needleham, et al. (1970) J. Mol. Biol. 48:443-453; Sankoff, et al. (1983) Time Warps, String Edits, and Macromolecules: The Theory and Practice of Sequence Comparison Chapter One, Addison-Wesley, Reading, MA; and software packages from IntelliGenetics, Mountain View, CA; and the University of Wisconsin Genetics Computer Group, Madison, WI.

Nucleic acids encoding mammalian mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine proteins will typically hybridize to the nucleic acid sequence of SEQ ID NO: 1, 3, 5, or 7 under stringent conditions. For example, nucleic acids encoding mpf4, mCTAP3, m6Ckine, or h6Ckine chemokine proteins will normally hybridize to the nucleic acid of SEQ ID NO: 1, 3, 5, or 7 under stringent hybridization conditions. Generally, stringent conditions are selected to be about 10° C lower than the thermal melting point (Tm) for the probe sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typically, stringent conditions will be those in which the salt concentration is about 0.2 molar at pH 7 and the temperature is at least about 50° C. Other factors may significantly affect the stringency of hybridization, including, among others, base composition and size of the complementary strands, the presence of organic solvents such as formamide, and the extent of base mismatching. A preferred embodiment will include nucleic acids which will bind to disclosed sequences in 50% formamide and 200 mM NaCl at 42° C.

An isolated mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine DNA can be readily modified by nucleotide substitutions, nucleotide deletions, nucleotide insertions, and short inversions of nucleotide stretches. These modifications result in novel DNA sequences which encode mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine antigens, their derivatives, or

proteins having highly similar physiological, immunogenic, or antigenic activity.

Modified sequences can be used to produce mutant antigens or to enhance expression. Enhanced expression may involve gene amplification, increased transcription, increased translation, and other mechanisms. Such mutant mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine derivatives include predetermined or site-specific mutations of the respective protein or its fragments. "Mutant mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine" encompasses a polypeptide otherwise falling within the homology definition of the human mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine as set forth above, but having an amino acid sequence which differs from that of a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine as found in nature, whether by way of deletion, substitution, or insertion. In particular, "site specific mutant mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine" generally includes proteins having significant similarity with a protein having a sequence of SEQ ID NO: 2, 4, 6, 8, or 10, e.g., natural embodiments, and as sharing various biological activities, e.g., antigenic or immunogenic, with those sequences, and in preferred embodiments contain most or all of the disclosed sequence. This applies also to polymorphic variants from different individuals. Similar concepts apply to different mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine proteins, particularly those found in various warm blooded animals, e.g., mammals and birds. As stated before, it is emphasized that descriptions are generally meant to encompass other mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine proteins, not limited to the mouse or human embodiments specifically discussed.

Although site specific mutation sites are predetermined, mutants need not be site specific. mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine mutagenesis can be conducted by making amino acid insertions or deletions. Substitutions, deletions,

insertions, or any combinations may be generated to arrive at a final construct. Insertions include amino- or carboxyl- terminal fusions, e.g. epitope tags. Random mutagenesis can be conducted at a target codon and the expressed mutants can then be screened for the desired activity. Methods for making substitution mutations at predetermined sites in DNA having a known sequence are well known in the art, e.g., by M13 primer mutagenesis or polymerase chain reaction (PCR) techniques. See also, Sambrook, et al. (1989) and Ausubel, et al. (1987 and Supplements). The mutations in the DNA normally should not place coding sequences out of reading frames and preferably will not create complementary regions that could hybridize to produce secondary mRNA structure such as loops or hairpins.

The present invention also provides recombinant proteins, e.g., heterologous fusion proteins using segments from these proteins. A heterologous fusion protein is a fusion of proteins or segments which are naturally not normally fused in the same manner. Thus, the fusion product of an immunoglobulin with a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine polypeptide is a continuous protein molecule having sequences fused in a typical peptide linkage, typically made as a single translation product and exhibiting properties derived from each source peptide. A similar concept applies to heterologous nucleic acid sequences.

In addition, new constructs may be made from combining similar functional domains from other proteins. For example, protein-binding or other segments may be "swapped" between different new fusion polypeptides or fragments. See, e.g., Cunningham, et al. (1989) Science 243:1330-1336; and O'Dowd, et al. (1988) J. Biol. Chem. 263:15985-15992. Thus, new chimeric polypeptides exhibiting new combinations of specificities will result from the functional linkage of protein-binding specificities and other functional domains.

VIII. Binding Agent:mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine Protein Complexes

A mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein that specifically, or selectively, binds to or that is specifically immunoreactive with an antibody generated against a defined immunogen, such as an immunogen consisting of the amino acid sequence of SEQ ID NO: 2, 4, 6, 8, or 10, is typically determined in an immunoassay. The immunoassay uses a polyclonal antiserum which was raised to a protein of SEQ ID NO: 2, 4, 6, 8, or 10. This antiserum is selected to have low crossreactivity against other chemokines and any such crossreactivity is removed by immunoabsorbtion prior to use in the immunoassay.

In order to produce antisera for use in an immunoassay, the protein of SEQ ID NO: 2, 4, 6, 8, or 10, is isolated as described herein. For example, recombinant protein may be produced in a mammalian cell line. An inbred strain of mice such as balb/c is immunized with the protein of SEQ ID NO: 2, 4, 6, 8, or 10, using a standard adjuvant, such as Freund's adjuvant, and a standard mouse immunization protocol (see Harlow and Lane, supra). Alternatively, a synthetic peptide, preferably near full length, derived from the sequences disclosed herein and conjugated to a carrier protein can be used an immunogen. Polyclonal sera are collected and titered against the immunogen protein in an immunoassay, for example, a solid phase immunoassay with the immunogen immobilized on a solid support. Polyclonal antisera with a titer of 10^4 or greater are selected and tested for their cross reactivity against C, CC, CX3C, and CXC chemokines, using a competitive binding immunoassay such as the one described in Harlow and Lane, supra, at pages 570-573. Preferably two chemokines are used in this determination in conjunction with human mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine.

Immunoassays in the competitive binding format can be used for the crossreactivity determinations. For example, a protein of SEQ ID NO: 2, 4, 6, 8, or 10 can be

immobilized to a solid support. Proteins added to the assay compete with the binding of the antisera to the immobilized antigen. The ability of the above proteins to compete with the binding of the antisera to the immobilized protein is compared to the protein of SEQ ID NO: 2, 4, 6, 8, or 10. The percent crossreactivity for the above proteins is calculated, using standard calculations. Those antisera with less than 10% crossreactivity with each of the proteins listed above are selected and pooled. The cross-reacting antibodies are then removed from the pooled antisera by immunoabsorbtion with the above-listed proteins.

The immunoabsorbed and pooled antisera are then used in a competitive binding immunoassay as described above to compare a second protein to the immunogen protein (e.g., the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine motif of SEQ ID NO: 2, 4, 6, 8, or 10). In order to make this comparison, the two proteins are each assayed at a wide range of concentrations and the amount of each protein required to inhibit 50% of the binding of the antisera to the immobilized protein is determined. If the amount of the second protein required is less than twice the amount of the protein, e.g., of SEQ ID NO: 2, 4, 6, 8, or 10 that is required, then the second protein is said to specifically bind to an antibody generated to the immunogen.

It is understood that each of mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine proteins are members of respective families of homologous proteins that comprise two or more genes. For a particular gene product, such as the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein, the term refers not only to the amino acid sequences disclosed herein, but also to other proteins that are polymorphic, allelic, or non-allelic variants. It is also understood that the term "mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine" includes nonnatural mutations introduced by deliberate mutation using conventional recombinant technology such as single site mutation, or by excising short sections of DNA

encoding mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine proteins, or by substituting new amino acids, or adding new amino acids. Such minor alterations should substantially maintain the immunoidentity of the original molecule and/or its biological activity. Thus, these alterations include proteins that are specifically immunoreactive with a designated naturally occurring mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein, for example, the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine protein shown in SEQ ID NO: 2, 4, 6, 8, or 10. The biological properties of the altered proteins can be determined by expressing the protein in an appropriate cell line and measuring, e.g., a chemotactic effect. Particular protein modifications considered minor would include conservative substitution of amino acids with similar chemical properties, as described above for the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine as a whole. By aligning a protein optimally with the protein of SEQ ID NO: 2, 4, 6, 8, or 10, and by using the conventional immunoassays described herein to determine immunoidentity, or by using lymphocyte chemotaxis assays, one can determine the protein compositions of the invention.

IX. Functional Variants

The blocking of physiological response to mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine may result from the inhibition of binding of the protein to its receptor, e.g., through competitive inhibition. Thus, in vitro assays of the present invention will often use isolated protein, membranes from cells expressing a recombinant membrane associated mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine, soluble fragments comprising receptor binding segments of these proteins, or fragments attached to solid phase substrates. These assays will also allow for the diagnostic determination of the effects of either binding segment mutations and modifications, or protein mutations and modifications, e.g., protein analogs. This invention also contemplates

the use of competitive drug screening assays, e.g., where neutralizing antibodies to antigen or receptor fragments compete with a test compound for binding to the protein. In this manner, the antibodies can be used to detect the presence of a polypeptide which shares one or more antigenic binding sites of the protein and can also be used to occupy binding sites on the protein that might otherwise interact with a receptor.

"Derivatives" of mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine antigens include amino acid sequence mutants, glycosylation variants, and covalent or aggregate conjugates with other chemical moieties. Covalent derivatives can be prepared by linkage of functionalities to groups which are found in mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine amino acid side chains or at the N- or C- termini, by means which are well known in the art. These derivatives can include, without limitation, aliphatic esters or amides of the carboxyl terminus, or of residues containing carboxyl side chains, O-acyl derivatives of hydroxyl group-containing residues, and N-acyl derivatives of the amino terminal amino acid or amino-group containing residues, e.g., lysine or arginine. Acyl groups are selected from the group of alkyl-moieties including C3 to C18 normal alkyl, thereby forming alkanoyl aroyl species. Covalent attachment to carrier proteins may be important when immunogenic moieties are haptens.

In particular, glycosylation alterations are included, e.g., made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing, or in further processing steps. Particularly preferred means for accomplishing this are by exposing the polypeptide to glycosylating enzymes derived from cells which normally provide such processing, e.g., mammalian glycosylation enzymes. Deglycosylation enzymes are also contemplated. Also embraced are versions of the same primary amino acid sequence which have other minor modifications, including phosphorylated amino acid residues, e.g., phosphotyrosine, phosphoserine, or

phosphothreonine, or other moieties, including ribosyl groups or cross-linking reagents.

A major group of derivatives are covalent conjugates of the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine or fragments thereof with other proteins or polypeptides. These derivatives can be synthesized in recombinant culture such as N- or C-terminal fusions or by the use of agents known in the art for their usefulness in cross-linking proteins through reactive side groups. Preferred protein derivatization sites with cross-linking agents are at free amino groups, carbohydrate moieties, and cysteine residues.

Fusion polypeptides between mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine and other homologous or heterologous proteins are also provided. Many growth factors and cytokines are homodimeric entities, and a repeat construct may have various advantages, including lessened susceptibility to proteolytic degradation. Moreover, many receptors require dimerization to transduce a signal, and various dimeric proteins or domain repeats can be desirable. Heterologous polypeptides may be fusions between different surface markers, resulting in, e.g., a hybrid protein exhibiting receptor binding specificity. Likewise, heterologous fusions may be constructed which would exhibit a combination of properties or activities of the derivative proteins. Typical examples are fusions of a reporter polypeptide, e.g., luciferase, with a segment or domain of a protein, e.g., a receptor-binding segment, so that the presence or location of the fused protein may be easily determined. See, e.g., Dull, et al., U.S. Patent No. 4,859,609. Other gene fusion partners include bacterial β-galactosidase, trpE, Protein A, ß-lactamase, alpha amylase, alcohol dehydrogenase, and yeast alpha mating factor. See, e.g., Godowski, et al. (1988) Science 241:812-816.

Such polypeptides may also have amino acid residues which have been chemically modified by phosphorylation, sulfonation, biotinylation, or the addition or removal of

other moieties, particularly those which have molecular shapes similar to phosphate groups. In some embodiments, the modifications will be useful labeling reagents, or serve as purification targets, e.g., affinity ligands.

This invention also contemplates the use of derivatives of mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine other than variations in amino acid sequence or glycosylation. Such derivatives may involve covalent or aggregative association with chemical moieties. These derivatives generally fall into the three classes: (1) salts, (2) side chain and terminal residue covalent modifications, and (3) adsorption complexes, for example with cell membranes. Such covalent or aggregative derivatives are useful as immunogens, as reagents in immunoassays, or in purification methods such as for affinity purification of ligands or other binding ligands. For example, a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine antigen can be immobilized by covalent bonding to a solid support such as cyanogen bromide-activated SEPHAROSE, by methods which are well known in the art, or adsorbed onto polyolefin surfaces, with or without glutaraldehyde cross-linking, for use in the assay or purification of anti-mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine antibodies or its receptor. The mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine can also be labeled with a detectable group, e.g., radioiodinated by the chloramine T procedure, covalently bound to rare earth chelates, or conjugated to another fluorescent moiety for use in diagnostic assays. Purification of mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokines may be effected by immobilized antibodies or receptor.

Isolated mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine genes will allow transformation of cells lacking expression of corresponding mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine, e.g., either species types or cells which lack corresponding proteins and exhibit negative background activity. Expression of transformed genes will allow isolation of antigenically

pure cell lines, with defined or single specie variants. This approach will allow for more sensitive detection and discrimination of the physiological effects of mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine receptor proteins. Subcellular fragments, e.g., cytoplasts or membrane fragments, can be isolated and used.

X. Uses

The present invention provides reagents which will find use in diagnostic applications as described elsewhere herein, e.g., in the general description for developmental abnormalities, or below in the description of kits for diagnosis.

mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine nucleotides, e.g., mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine DNA or RNA, may be used as a component in a forensic assay. For instance, the nucleotide sequences provided may be labeled using, e.g., ^{32}P or biotin and used to probe standard restriction fragment polymorphism blots, providing a measurable character to aid in distinguishing between individuals. Such probes may be used in well-known forensic techniques such as genetic fingerprinting. In addition, nucleotide probes made from mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine sequences may be used in *in situ* assays to detect chromosomal abnormalities. For instance, rearrangements in the human chromosome encoding a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine gene may be detected via well-known in situ techniques, using mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine probes in conjunction with other known chromosome markers.

Antibodies and other binding agents directed towards mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine proteins or nucleic acids may be used to purify the corresponding mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine molecule. As described in the Examples below, antibody purification of mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine components is

both possible and practicable. Antibodies and other binding agents may also be used in a diagnostic fashion to determine whether mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine components are present in a tissue sample or cell population using well-known techniques described herein. The ability to attach a binding agent to a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine provides a means to diagnose disorders associated with mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine misregulation. Antibodies and other mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine binding agents may also be useful as histological markers. As described in the examples below, mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine expression is limited to specific tissue types. By directing a probe, such as an antibody or nucleic acid to a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine it is possible to use the probe to distinguish tissue and cell types in situ or in vitro.

This invention also provides reagents with significant therapeutic value. The mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine (naturally occurring or recombinant), fragments thereof, and antibodies thereto, along with compounds identified as having binding affinity to a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine, are useful in the treatment of conditions associated with abnormal physiology or development, including abnormal proliferation, e.g., cancerous conditions, or degenerative conditions. Abnormal proliferation, regeneration, degeneration, and atrophy may be modulated by appropriate therapeutic treatment using the compositions provided herein. For example, a disease or disorder associated with abnormal expression or abnormal signaling by a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine is a target for an agonist or antagonist of the protein. The proteins likely play a role in regulation or development of neuronal or

hematopoietic cells, e.g., lymphoid cells, which affect immunological responses.

In particular, an antagonist for h6Ckine may have potential for T cell mediated autoimmunity, e.g., joint inflammation, organ/tissue inflammation. The fact that h6Ckine is expressed in secondary lymphoid organs, e.g., lymph nodes, tonsils, indicates that h6Ckine may be a preliminary molecule involved in the initial inflammatory response.

Other abnormal developmental conditions are known in cell types shown to possess mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine mRNA by northern blot analysis. See Berkow (ed.) The Merck Manual of Diagnosis and Therapy, Merck & Co., Rahway, NJ; and Thorn, et al. Harrison's Principles of Internal Medicine, McGraw-Hill, NY. Developmental or functional abnormalities, e.g., of the neuronal or immune system, cause significant medical abnormalities and conditions which may be susceptible to prevention or treatment using compositions provided herein.

Certain chemokines have also been implicated in viral replication mechanisms. See, e.g., Cohen (1996) Science 272:809-810; Feng, et al. (1996) Science 272:872-877; and Cocchi, et al. (1995) Science 270:1811-1816. The mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine may be useful in a similar context. Alternatively, the stalk structure may be very important in presentation of the ligand domain, and other chemokines may be advantageously substituted for the chemokine domain in this molecule.

Recombinant mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine or mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine antibodies can be purified and then administered to a patient. These reagents can be combined for therapeutic use with additional active or inert ingredients, e.g., in conventional pharmaceutically acceptable carriers or diluents, e.g., immunogenic adjuvants, along with physiologically innocuous stabilizers and excipients. These combinations can be

sterile filtered and placed into dosage forms as by lyophilization in dosage vials or storage in stabilized aqueous preparations. This invention also contemplates use of antibodies or binding fragments thereof, including forms which are not complement binding.

Drug screening using antibodies or receptor or fragments thereof can identify compounds having binding affinity to mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine, including isolation of associated components. Subsequent biological assays can then be utilized to determine if the compound has intrinsic stimulating activity and is therefore a blocker or antagonist in that it blocks the activity of the protein. Likewise, a compound having intrinsic stimulating activity can activate the receptor and is thus an agonist in that it simulates the activity of a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine. This invention further contemplates the therapeutic use of antibodies to mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine as antagonists. This approach should be particularly useful with other mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine species variants.

The quantities of reagents necessary for effective therapy will depend upon many different factors, including means of administration, target site, physiological state of the patient, and other medicants administered. Thus, treatment dosages should be titrated to optimize safety and efficacy. Typically, dosages used in vitro may provide useful guidance in the amounts useful for in situ administration of these reagents. Animal testing of effective doses for treatment of particular disorders will provide further predictive indication of human dosage. Various considerations are described, e.g., in Gilman, et al. (eds.) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics (8th ed.) Pergamon Press; and (1990) Remington's Pharmaceutical Sciences (17th ed.) Mack Publishing Co., Easton, PA. Methods for administration are discussed therein and below, e.g., for oral, intravenous,

intraperitoneal, or intramuscular administration, transdermal diffusion, and others. Pharmaceutically acceptable carriers will include water, saline, buffers, and other compounds described, e.g., in the Merck Index, Merck & Co., Rahway, NJ. Dosage ranges would ordinarily be expected to be in amounts lower than 1 mM concentrations, typically less than about 10 µM concentrations, usually less than about 100 nM, preferably less than about 10 pM (picomolar), and most preferably less than about 1 fM (femtomolar), with an appropriate carrier. Slow release formulations, or a slow release apparatus will often be utilized for continuous administration.

mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokines, fragments thereof, and antibodies to it or its fragments, antagonists, and agonists, may be administered directly to the host to be treated or, depending on the size of the compounds, it may be desirable to conjugate them to carrier proteins such as ovalbumin or serum albumin prior to their administration. Therapeutic formulations may be administered in any conventional dosage formulation. While it is possible for the active ingredient to be administered alone, it is preferable to present it as a pharmaceutical formulation. Formulations typically comprise at least one active ingredient, as defined above, together with one or more acceptable carriers thereof. Each carrier should be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the patient. Formulations include those suitable for oral, rectal, nasal, or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. See, e.g., Gilman, et al. (eds.) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics (8th ed.) Pergamon Press; and (1990) Remington's Pharmaceutical Sciences (17th ed.) Mack

Publishing Co., Easton, PA; Avis, et al. (eds.) (1993) Pharmaceutical Dosage Forms: Parenteral Medications Dekker, NY; Lieberman, et al. (eds.) (1990) Pharmaceutical Dosage Forms: Tablets Dekker, NY; and Lieberman, et al. (eds.) (1990) Pharmaceutical Dosage Forms: Disperse Systems Dekker, NY. The therapy of this invention may be combined with or used in association with other therapeutic agents.

Both the naturally occurring and the recombinant forms of the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokines of this invention are particularly useful in kits and assay methods which are capable of screening compounds for binding activity to the proteins. Several methods of automating assays have been developed in recent years so as to permit screening of tens of thousands of compounds in a short period. See, e.g., Fodor, et al. (1991) Science 251:767-773, and other descriptions of chemical diversity libraries, which describe means for testing of binding affinity by a plurality of compounds. The development of suitable assays can be greatly facilitated by the availability of large amounts of purified, soluble mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine as provided by this invention.

For example, antagonists can normally be found once the protein has been structurally defined. Testing of potential protein analogs is now possible upon the development of highly automated assay methods using a purified receptor. In particular, new agonists and antagonists will be discovered by using screening techniques described herein. Of particular importance are compounds found to have a combined binding affinity for multiple mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine receptors, e.g., compounds which can serve as antagonists for species variants of a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine.

This invention is particularly useful for screening compounds by using recombinant protein in a variety of drug screening techniques. The advantages of using a

recombinant protein in screening for specific ligands include: (a) improved renewable source of the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine from a specific source; (b) potentially greater number of ligands per cell giving better signal to noise ratio in assays; and (c) species variant specificity (theoretically giving greater biological and disease specificity).

One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant DNA molecules expressing a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine receptor, e.g., CXCR3 for 6Ckine. Cells may be isolated which express a receptor in isolation from any others. Such cells, either in viable or fixed form, can be used for standard ligand/receptor binding assays. See also, Parce, et al. (1989) Science 246:243-247; and Owicki, et al. (1990) Proc. Nat'l Acad. Sci. USA 87:4007-4011, which describe sensitive methods to detect cellular responses.

Competitive assays are particularly useful, where the cells (source of mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine) are contacted and incubated with a labeled receptor or antibody having known binding affinity to the ligand, such as ^{125}I-antibody, and a test sample whose binding affinity to the binding composition is being measured. The bound and free labeled binding compositions are then separated to assess the degree of ligand binding. The amount of test compound bound is inversely proportional to the amount of labeled receptor binding to the known source. Any one of numerous techniques can be used to separate bound from free ligand to assess the degree of ligand binding. This separation step could typically involve a procedure such as adhesion to filters followed by washing, adhesion to plastic followed by washing, or centrifugation of the cell membranes. Viable cells could also be used to screen for the effects of drugs on mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine mediated functions, e.g., second messenger levels, i.e., Ca^{++}; cell proliferation;

inositol phosphate pool changes; and others. Some detection methods allow for elimination of a separation step, e.g., a proximity sensitive detection system. Calcium sensitive dyes will be useful for detecting Ca^{++} levels, with a fluorimeter or a fluorescence cell sorting apparatus.

The 6Ckine-CXCR3 ligand receptor pair can also be used as a positive control to identify other CXCR3 ligands. For example, CXCR3 can be attached to a solid support, e.g., a chip, a bead in an affinity column, a microtiter plate well. Unknown samples containing potential ligands are incubated with the receptor. 6Ckine is treated similarly in a separate sample. Binding affinities are measured as described above and compared to 6Ckine. Similarly, cells expressing CXCR3, which respond biologically to ligand binding, can be assayed and responses compared to 6Ckine induced activity.

Another method utilizes membranes from transformed eukaryotic or prokaryotic host cells as the source of a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine. These cells are stably transformed with DNA vectors directing the expression of a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine, e.g., an engineered membrane bound form. Essentially, the membranes would be prepared from the cells and used in a receptor/ligand binding assay such as the competitive assay set forth above.

Still another approach is to use solubilized, unpurified or solubilized, purified mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine from transformed eukaryotic or prokaryotic host cells. This allows for a "molecular" binding assay with the advantages of increased specificity, the ability to automate, and high drug test throughput.

Another technique for drug screening involves an approach which provides high throughput screening for compounds having suitable binding affinity to a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine antibody

and is described in detail in Geysen, European Patent Application 84/03564, published on September 13, 1984. First, large numbers of different small peptide test compounds are synthesized on a solid substrate, e.g., plastic pins or some other appropriate surface, see Fodor, et al., supra. Then all the pins are reacted with solubilized, unpurified or solubilized, purified mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine antibody, and washed. The next step involves detecting bound mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine antibody.

Rational drug design may also be based upon structural studies of the molecular shapes of the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine and other effectors or analogs. See, e.g., Methods in Enzymology vols. 202 and 203. Effectors may be other proteins which mediate other functions in response to ligand binding, or other proteins which normally interact with the receptor. One means for determining which sites interact with specific other proteins is a physical structure determination, e.g., x-ray crystallography or 2 dimensional NMR techniques. These will provide guidance as to which amino acid residues form molecular contact regions. For a detailed description of protein structural determination, see, e.g., Blundell and Johnson (1976) Protein Crystallography Academic Press, NY.

A purified mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine can be coated directly onto plates for use in the aforementioned drug screening techniques. However, non-neutralizing antibodies to these ligands can be used as capture antibodies to immobilize the respective ligand on the solid phase.

XI. Kits

This invention also contemplates use of mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine proteins, fragments thereof, peptides, and their fusion products in a variety of diagnostic kits and methods for detecting the presence of mpf4, mCTAP3, m6Ckine, h6Ckine,

or Chr19kine chemokine or a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine receptor. Typically the kit will have a compartment containing either a defined mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine peptide or gene segment or a reagent which recognizes one or the other, e.g., receptor fragments or antibodies.

A kit for determining the binding affinity of a test compound to a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine would typically comprise a test compound; a labeled compound, e.g., a receptor or antibody having known binding affinity for the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine; a source of mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine (naturally occurring or recombinant); and a means for separating bound from free labeled compound, such as a solid phase for immobilizing the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine. Once compounds are screened, those having suitable binding affinity to the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine can be evaluated in suitable biological assays, as are well known in the art, to determine whether they act as agonists or antagonists to the receptor. The availability of recombinant mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine polypeptides also provide well defined standards for calibrating such assays.

A preferred kit for determining the concentration of, for example, a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine in a sample would typically comprise a labeled compound, e.g., receptor or antibody, having known binding affinity for the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine, a source of mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine (naturally occurring or recombinant), and a means for separating the bound from free labeled compound, for example, a solid phase for immobilizing the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine. Compartments containing reagents, and instructions, will normally be provided.

Antibodies, including antigen binding fragments, specific for the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine or ligand fragments are useful in diagnostic applications to detect the presence of elevated levels of mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine and/or its fragments. Such diagnostic assays can employ lysates, live cells, fixed cells, immunofluorescence, cell cultures, body fluids, and further can involve the detection of antigens related to the ligand in serum, or the like. Diagnostic assays may be homogeneous (without a separation step between free reagent and antigen-mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine complex) or heterogeneous (with a separation step). Various commercial assays exist, such as radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), enzyme-multiplied immunoassay technique (EMIT), substrate-labeled fluorescent immunoassay (SLFIA), and the like. For example, unlabeled antibodies can be employed by using a second antibody which is labeled and which recognizes the antibody to a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine or to a particular fragment thereof. Similar assays have also been extensively discussed in the literature. See, e.g., Harlow and Lane (1988) Antibodies: A Laboratory Manual, CSH Press, NY; Chan (ed.) (1987) Immunoassay: A Practical Guide Academic Press, Orlando, FL; Price and Newman (eds.) (1991) Principles and Practice of Immunoassay Stockton Press, NY; and Ngo (ed.) (1988) Nonisotopic Immunoassay Plenum Press, NY.

Anti-idiotypic antibodies may have similar use to diagnose presence of antibodies against a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine, as such may be diagnostic of various abnormal states. For example, overproduction of mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine may result in production of various immunological or other medical reactions which may be diagnostic of abnormal physiological states, e.g., in cell growth, activation, or differentiation.

Frequently, the reagents for diagnostic assays are supplied in kits, so as to optimize the sensitivity of the assay. For the subject invention, depending upon the nature of the assay, the protocol, and the label, either labeled or unlabeled antibody or receptor, or labeled mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine is provided. This is usually in conjunction with other additives, such as buffers, stabilizers, materials necessary for signal production such as substrates for enzymes, and the like. Preferably, the kit will also contain instructions for proper use and disposal of the contents after use. Typically the kit has compartments for each useful reagent. Desirably, the reagents are provided as a dry lyophilized powder, where the reagents may be reconstituted in an aqueous medium providing appropriate concentrations of reagents for performing the assay.

Many of the aforementioned constituents of the drug screening and the diagnostic assays may be used without modification, or may be modified in a variety of ways. For example, labeling may be achieved by covalently or non-covalently joining a moiety which directly or indirectly provides a detectable signal. In any of these assays, the protein, test compound, mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine, or antibodies thereto can be labeled either directly or indirectly. Possibilities for direct labeling include label groups: radiolabels such as ^{125}I, enzymes (U.S. Pat. No. 3,645,090) such as peroxidase and alkaline phosphatase, and fluorescent labels (U.S. Pat. No. 3,940,475) capable of monitoring the change in fluorescence intensity, wavelength shift, or fluorescence polarization. Possibilities for indirect labeling include biotinylation of one constituent followed by binding to avidin coupled to one of the above label groups.

There are also numerous methods of separating the bound from the free ligand, or alternatively the bound from the free test compound. The mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine can be immobilized on

various matrices followed by washing. Suitable matrices include plastic such as an ELISA plate, filters, and beads. Methods of immobilizing the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine to a matrix include, without limitation, direct adhesion to plastic, use of a capture antibody, chemical coupling, and biotin-avidin. The last step in this approach involves the precipitation of ligand/receptor or ligand/antibody complex by any of several methods including those utilizing, e.g., an organic solvent such as polyethylene glycol or a salt such as ammonium sulfate. Other suitable separation techniques include, without limitation, the fluorescein antibody magnetizable particle method described in Rattle, et al. (1984) Clin. Chem. 30:1457-1461, and the double antibody magnetic particle separation as described in U.S. Pat. No. 4,659,678.

Methods for linking proteins or their fragments to the various labels have been extensively reported in the literature and do not require detailed discussion here. Many of the techniques involve the use of activated carboxyl groups either through the use of carbodiimide or active esters to form peptide bonds, the formation of thioethers by reaction of a mercapto group with an activated halogen such as chloroacetyl, or an activated olefin such as maleimide, for linkage, or the like. Fusion proteins will also find use in these applications.

Another diagnostic aspect of this invention involves use of oligonucleotide or polynucleotide sequences taken from the sequence of a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine. These sequences can be used as probes for detecting levels of the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine message in samples from natural sources, or patients suspected of having an abnormal condition, e.g., cancer or developmental problem. The preparation of both RNA and DNA nucleotide sequences, the labeling of the sequences, and the preferred size of the sequences has received ample description and discussion in the literature. Normally

an oligonucleotide probe should have at least about 14 nucleotides, usually at least about 18 nucleotides, and the polynucleotide probes may be up to several kilobases. Various labels may be employed, most commonly radionuclides, particularly ^{32}P. However, other techniques may also be employed, such as using biotin modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorophores, enzymes, or the like. Alternatively, antibodies may be employed which can recognize specific duplexes, including DNA duplexes, RNA duplexes, DNA-RNA hybrid duplexes, or DNA-protein duplexes. The antibodies in turn may be labeled and the assay carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected. The use of probes to the novel anti-sense RNA may be carried out using many conventional techniques such as nucleic acid hybridization, plus and minus screening, recombinational probing, hybrid released translation (HRT), and hybrid arrested translation (HART). This also includes amplification techniques such as polymerase chain reaction (PCR).

Diagnostic kits which also test for the qualitative or quantitative presence of these and other markers are also contemplated. Diagnosis or prognosis may depend on the combination of multiple indications used as markers. Thus, kits may test for combinations of markers. See, e.g., Viallet, et al. (1989) Progress in Growth Factor Res. 1:89-97. Qualitative or quantitative expression of each chemokine may be evaluated by standard methods at the protein or mRNA levels.

XII. Receptor Isolation

Having isolated a binding partner of a specific interaction, methods exist for isolating the counter-partner. See, Gearing, et al. (1989) EMBO J. 8:3667-

3676. For example, means to label a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine without interfering with the binding to its receptor can be determined. For example, an affinity label or epitope tag can be fused to either the amino- or carboxyl-terminus of the ligand. An expression library can be screened for specific binding of the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine, e.g., by cell sorting, or other screening to detect subpopulations which express such a binding component. See, e.g., Ho, et al. (1993) Proc. Nat'l Acad. Sci. USA 90:11267-11271. Alternatively, a panning method may be used. See, e.g., Seed and Aruffo (1987) Proc. Nat'l Acad. Sci. USA 84:3365-3369. A two-hybrid selection system may also be applied making appropriate constructs with the available chemokine sequences. See, e.g., Fields and Song (1989) Nature 340:245-246. Standard Ca^{++} flux methods can also be utilized. See, e.g., Coligan, et al. (eds.) (1992 and periodic supplements) Current Protocols in Immunology Greene/Wiley, New York, NY.

Protein cross-linking techniques with label can be applied to isolate binding partners of a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine. This would allow identification of proteins which specifically interact with a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine, e.g., in a ligand-receptor like manner. Typically, the chemokine family binds to receptors of the seven transmembrane receptor family, and the receptor for the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine is likely to exhibit a similar structure. Thus, it is likely that the receptor will be found by expression in a system which is capable of expressing such a membrane protein in a form capable of exhibiting ligand binding capability.

The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the invention to specific embodiments.

EXAMPLES

I. General Methods

Many of the standard methods below are described or referenced, e.g., in Maniatis, et al. (1982) Molecular Cloning, A Laboratory Manual Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY; Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual (2d ed.) Vols. 1-3, CSH Press, NY; Ausubel, et al., Biology Greene Publishing Associates, Brooklyn, NY; or Ausubel, et al. (1987 and Supplements) Current Protocols in Molecular Biology Wiley/Greene, NY; Innis, et al. (eds.) (1990) PCR Protocols: A Guide to Methods and Applications Academic Press, NY. Methods for protein purification include such methods as ammonium sulfate precipitation, column chromatography, electrophoresis, centrifugation, crystallization, and others. See, e.g., Ausubel, et al. (1987 and periodic supplements); Deutscher (1990) "Guide to Protein Purification," Methods in Enzymology vol. 182, and other volumes in this series; and manufacturer's literature on use of protein purification products, e.g., Pharmacia, Piscataway, NJ, or Bio-Rad, Richmond, CA. Combination with recombinant techniques allow fusion to appropriate segments (epitope tags), e.g., to a FLAG sequence or an equivalent which can be fused, e.g., via a protease-removable sequence. See, e.g., Hochuli (1989) Chemische Industrie 12:69-70; Hochuli (1990) "Purification of Recombinant Proteins with Metal Chelate Absorbent" in Setlow (ed.) Genetic Engineering, Principle and Methods 12:87-98, Plenum Press, NY; and Crowe, et al. (1992) QIAexpress: The High Level Expression & Protein Purification System QUIAGEN, Inc., Chatsworth, CA.

Standard immunological techniques are described, e.g., in Coligan (1991) Current Protocols in Immunology Wiley/Greene, NY; and Methods in Enzymology volumes. 70, 73, 74, 84, 92, 93, 108, 116, 121, 132, 150, 162, and 163. Assays for neural cell biological activities are described, e.g., in Wouterlood (ed. 1995) Neuroscience Protocols modules 10, Elsevier; Methods in Neurosciences

Academic Press; and Neuromethods Humana Press, Totowa, NJ. Methodology of developmental systems is described, e.g., in Meisami (ed.) Handbook of Human Growth and Developmental Biology CRC Press; and Chrispeels (ed.) Molecular Techniques and Approaches in Developmental Biology Interscience.

FACS analyses are described in Melamed, et al. (1990) Flow Cytometry and Sorting Wiley-Liss, Inc., New York, NY; Shapiro (1988) Practical Flow Cytometry Liss, New York, NY; and Robinson, et al. (1993) Handbook of Flow Cytometry Methods Wiley-Liss, New York, NY.

II. Isolation of mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine clone

A clone encoding the mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine is isolated from a natural source by many different possible methods. Given the sequences provided herein, PCR primers or hybridization probes are selected and/or constructed to isolate either genomic DNA segments or cDNA reverse transcripts. Appropriate cell sources include listed tissues, e.g., brain libraries. Tissue distribution below also suggests source tissues. Genetic and polymorphic or allelic variants are isolated by screening a population of individuals.

PCR based detection is performed by standard methods, preferably using primers from opposite ends of the coding sequence, but flanking segments might be selected for specific purposes.

Alternatively, hybridization probes are selected. Particular AT or GC contents of probes are selected depending upon the expected homology and mismatching expected. Appropriate stringency conditions are selected to balance an appropriate positive signal to background ratio. Successive washing steps are used to collect clones of greater homology.

Further clones are isolated using an antibody based selection procedure. Standard expression cloning methods are applied including, e.g., FACS staining of membrane associated expression product. The antibodies are used

to identify clones producing a recognized protein. Alternatively, antibodies are used to purify a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine, with protein sequencing and standard means to isolate a gene encoding that protein.

Genomic sequence based methods will also allow for identification of sequences naturally available, or otherwise, which exhibit homology to the provided sequences.

III. Isolation of a primate counterpart for mpf4, mCTAP3, 6Ckine, or Chr19kine chemokine clone

Similar methods are used as above to isolate an appropriate primate chemokine gene from another primate. Similar source materials as indicated above are used to isolate natural genes, including genetic, polymorphic, allelic, or strain variants. Other species variants are also isolated using similar methods. Alternatively, gene databases may be searched for the appropriate motifs.

IV. Isolation of a rodent pf4, CTAP3, 6Ckine, or Chr19kine chemokine clone

An appropriate rodent source is selected as above, e.g., rat, hamster, etc. Similar methods are utilized to isolate a species variant, though the level of similarity will typically be lower for rodent chemokine as compared to a human to other primate sequence.

V. Expression; purification; characterization

With an appropriate clone from above, the coding sequence is inserted into an appropriate expression vector. This may be in a vector specifically selected for a prokaryote, yeast, insect, or higher vertebrate, e.g., mammalian expression system. Standard methods are applied to produce the gene product, preferably as a soluble secreted molecule, but will, in certain instances, also be made as an intracellular protein. Intracellular proteins typically require cell lysis to

recover the protein, and insoluble inclusion bodies are a common starting material for further purification.

With a clone encoding a mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine, recombinant production means are used, although natural forms may be purified from appropriate sources. The protein product is purified by standard methods of protein purification, in certain cases, e.g., coupled with immunoaffinity methods. Immunoaffinity methods are used either as a purification step, as described above, or as a detection assay to determine the separation properties of the protein.

Preferably, the protein is secreted into the medium, and the soluble product is purified from the medium in a soluble form. Alternatively, as described above, inclusion bodies from prokaryotic expression systems are a useful source of material. Typically, the insoluble protein is solubilized from the inclusion bodies and refolded using standard methods. Purification methods are developed as described above.

The product of the purification method described above is characterized to determine many structural features. Standard physical methods are applied, e.g., amino acid analysis and protein sequencing. The resulting protein is subjected to CD spectroscopy and other spectroscopic methods, e.g., NMR, ESR, mass spectroscopy, etc. The product is characterized to determine its molecular form and size, e.g., using gel chromatography and similar techniques. Understanding of the chromatographic properties will lead to more gentle or efficient purification methods.

Prediction of glycosylation sites may be made, e.g., as reported in Hansen, et al. (1995) Biochem. J. 308:801-813.

VI. Preparation of antibodies against mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine

With DNA for expression, or protein produced, e.g., as above, animals are immunized to produce antibodies. Polyclonal antiserum is raised, in some cases, using non-

purified antigen, though the resulting serum will exhibit higher background levels. Preferably, the antigen is purified using standard protein purification techniques, including, e.g., affinity chromatography using polyclonal serum indicated above. Presence of specific antibodies is detected using defined synthetic peptide fragments.

Polyclonal serum is raised against a purified antigen, purified as indicated above, or using, e.g., a plurality of, synthetic peptides. A series of overlapping synthetic peptides which encompass all of the full length sequence, if presented to an animal, will produce serum recognizing most linear epitopes on the protein. Such an antiserum is used to affinity purify protein, which is, in turn, used to introduce intact full length protein into another animal to produce another antiserum preparation.

Similar techniques are used to generate induce monoclonal antibodies to either unpurified antigen, or, preferably, purified antigen. The antiserum or antibodies may recognize native protein, or may recognize denatured antigen.

VII. Cellular and tissue distribution

Distribution of the protein or gene products are determined, e.g., using immunohistochemistry with an antibody reagent, as produced above, or by screening for nucleic acids encoding the chemokine. Hybridization or PCR methods are used to detect DNA, cDNA, or message content. Histochemistry allows determination of the specific cell types within a tissue which express higher or lower levels of message or DNA. Antibody techniques are useful to quantitate protein in a biological sample, including a liquid or tissue sample. Immunoassays are developed to quantitate protein. Also, FACS analysis may be used to evaluate expression in a cell population.

VIII. Microchemotaxis assays

The pro-migratory activities of mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine are assessed in

microchemotaxis assays. See, e.g., Bacon, et al. (1988) Br. J. Pharmacol. 95:966-974.

Chemokines may also be assayed for activity in hemopoietic assays as described, e.g., by H. Broxmeyer. They may be assayed for angiogenic activities as described, e.g., by R. Streiter.

IX. Biological activities, direct and indirect

A robust and sensitive assay is selected as described above, e.g., on immune cells, neuronal cells, or stem cells. Chemokine is added to the assay in increasing doses to see if a dose response is detected. For example, in a proliferation assay, cells are plated out in plates. Appropriate culture medium is provided, and chemokine is added to the cells in varying amounts. Growth is monitored over a period of time which will detect either a direct effect on the cells, or an indirect effect of the chemokine.

Alternatively, an activation assay or attraction assay is used. An appropriate cell type is selected, e.g., hematopoietic cells, myeloid (macrophages, neutrophils, polymorphonuclear cells, etc.) or lymphoid (T cell, B cell, or NK cells), neural cells (neurons, neuroglia, oligodendrocytes, astrocytes, etc.), or stem cells, e.g., progenitor cells which differentiate to other cell types, e.g., gut crypt cells and undifferentiated cell types.

Other assays will be those which have been demonstrated with other chemokines. See, e.g., Schall and Bacon (1994) Current Opinion in Immunology 6:865-873; and Bacon and Schall (1996) Int. Arch. Allergy & Immunol. 109:97-109.

IX. Structure activity relationship

Information on the criticality of particular residues is determined using standard procedures and analysis. Standard mutagenesis analysis is performed, e.g., by generating many different variants at determined positions, e.g., at the positions identified above, and

evaluating biological activities of the variants. This may be performed to the extent of determining positions which modify activity, or to focus on specific positions to determine the residues which can be substituted to either retain, block, or modulate biological activity.

Alternatively, analysis of natural variants can indicate what positions tolerate natural mutations. This may result from populational analysis of variation among individuals, or across strains or species. Samples from selected individuals are analysed, e.g., by PCR analysis and sequencing. This allows evaluation of population polymorphisms.

X. Screening for agonists/antagonists

Agonists or antagonists are screened for ability to induce or block biological activity. The candidate compounds, e.g., sequence variants of natural mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine, are assayed for their biological activities. Alternatively, compounds are screened, alone or in combinations, to determine effects on biological activity.

XI. Isolation of a Receptor for mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine

A mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine can be used as a specific binding reagent to identify its binding partner, by taking advantage of its specificity of binding, much like an antibody would be used. A binding reagent is either labeled as described above, e.g., fluorescence or otherwise, or immobilized to a substrate for panning methods. The typical chemokine receptor is a seven transmembrane receptor.

The binding composition, e.g., chemokine, is used to screen an expression library made from a cell line which expresses a binding partner, i.e. receptor. Standard staining techniques are used to detect or sort intracellular or surface expressed receptor, or surface expressing transformed cells are screened by panning. Screening of intracellular expression is performed by

various staining or immunofluorescence procedures. See also McMahan, et al. (1991) EMBO J. 10:2821-2832.

Using standard Ca^{++} flux protocols, see, e.g., Coligan, et al. (eds.)(1992 and periodic supplements) Current Protocols in Immunol. Greene/Wiley, New York, NY, a receptor for m6Ckine was found to be a CXC chemokine receptor, designated CXCR3. This receptor is shared with IP-10 and Mig, chemokines which possess potent angiostatic and antitumor properties. See, e.g., Keane, et al. (1997) J. Immunol. 159:1437-1443; and Farber (1997) J. Leukoc. Biol. 61:246-257.

For example, on day 0, precoat 2-chamber permanox slides with 1 ml per chamber of fibronectin, 10 ng/ml in PBS, for 30 min at room temperature. Rinse once with PBS. Then plate COS cells at 2-3 x 10^5 cells per chamber in 1.5 ml of growth media. Incubate overnight at 37° C.

On day 1 for each sample, prepare 0.5 ml of a solution of 66 µg/ml DEAE-dextran, 66 µM chloroquine, and 4 µg DNA in serum free DME. For each set, a positive control is prepared, e.g., of human mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine chemokine cDNA at 1 and 1/200 dilution, and a negative mock. Rinse cells with serum free DME. Add the DNA solution and incubate 5 hr at 37° C. Remove the medium and add 0.5 ml 10% DMSO in DME for 2.5 min. Remove and wash once with DME. Add 1.5 ml growth medium and incubate overnight.

On day 2, change the medium. On days 3 or 4, the cells are fixed and stained. Rinse the cells twice with Hank's Buffered Saline Solution (HBSS) and fix in 4% paraformaldehyde (PFA)/glucose for 5 min. Wash 3X with HBSS. The slides may be stored at -80° C after all liquid is removed. For each chamber, 0.5 ml incubations are performed as follows. Add HBSS/saponin (0.1%) with 32 µl/ml of 1 M NaN_3 for 20 min. Cells are then washed with HBSS/saponin 1X. Add chemokine or chemokine/antibody complex to cells and incubate for 30 min. Wash cells twice with HBSS/saponin. If appropriate, add first antibody for 30 min. Add second antibody, e.g., Vector anti-mouse antibody, at 1/200

dilution, and incubate for 30 min. Prepare ELISA solution, e.g., Vector Elite ABC horseradish peroxidase solution, and preincubate for 30 min. Use, e.g., 1 drop of solution A (avidin) and 1 drop solution B (biotin) per 2.5 ml HBSS/saponin. Wash cells twice with HBSS/saponin. Add ABC HRP solution and incubate for 30 min. Wash cells twice with HBSS, second wash for 2 min, which closes cells. Then add Vector diaminobenzoic acid (DAB) for 5 to 10 min. Use 2 drops of buffer plus 4 drops DAB plus 2 drops of H_2O_2 per 5 ml of glass distilled water. Carefully remove chamber and rinse slide in water. Air dry for a few minutes, then add 1 drop of Crystal Mount and a cover slip. Bake for 5 min at 85-90° C.

Evaluate positive staining of pools and progressively subclone to isolation of single genes responsible for the binding.

Alternatively, chemokine reagents are used to affinity purify or sort out cells expressing a receptor. See, e.g., Sambrook, et al. or Ausubel, et al.

Another strategy is to screen for a membrane bound receptor by panning. The receptor cDNA is constructed as described above. The ligand can be immobilized and used to immobilize expressing cells. Immobilization may be achieved by use of appropriate antibodies which recognize, e.g., a FLAG sequence of a chemokine fusion construct, or by use of antibodies raised against the first antibodies. Recursive cycles of selection and amplification lead to enrichment of appropriate clones and eventual isolation of receptor expressing clones.

Phage expression libraries can be screened by chemokine. Appropriate label techniques, e.g., anti-FLAG antibodies, will allow specific labeling of appropriate clones.

XII. Immunohistochemical localization

The antibody described above is used to identify expression of mpf4, mCTAP3, m6Ckine, h6Ckine, or Chr19kine in various tissues. Methods for immunohistochemical staining are described, e.g., in

Sheehan, et al. (eds.) (1987) Theory and Practice of Histotechnology, Battelle Press, Columbus, OH.

All references cited herein are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

SEQUENCE SUBMISSION

SEQ ID NO: 1 is mpf4 mouse nucleic acid sequence.
SEQ ID NO: 2 is mpf4 mouse amino acid sequence.
SEQ ID NO: 3 is mCTAP3 mouse nucleic acid sequence.
SEQ ID NO: 4 is mCTAP3 mouse amino acid sequence.
SEQ ID NO: 5 is m6Ckine mouse nucleic acid sequence.
SEQ ID NO: 6 is m6Ckine mouse amino acid sequence.
SEQ ID NO: 7 is h6Ckine human nucleic acid sequence.
SEQ ID NO: 8 is h6Ckine human amino acid sequence.
SEQ ID NO: 9 is 6Ckine porcine amino acid sequence.
SEQ ID NO: 10 is Chr19kine human amino acid sequence.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Schering-Corp.

(ii) TITLE OF INVENTION: MAMMALIAN CHEMOKINES

(iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:
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(E) COUNTRY: USA
(F) ZIP: 07033-0530

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: Apple Macintosh
(C) OPERATING SYSTEM: Macintosh 7.5.3
(D) SOFTWARE: Microsoft Word 6.1

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 60/027,242
(B) FILING DATE: 02-OCT-1996

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 60/028,042
(B) FILING DATE: 09-OCT-1996

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 60/058,007
(B) FILING DATE: 28-AUG-1997

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 540 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 141..455

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 258..455

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CCTGGGTTTC CGGACTGGGC AGGCAGTGAA GATAAAACGT GCTTGGGAAG TCCCAGGAGC
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TGCTGGCCTG CACTTAAGAG CCCTAGACCC ATTTCCTCAA GGTAGAACTT TATCTTTGGG
120

TCCAGTGGCA CCCTCTTGAC ATG AGC GTC GCT GCG GTG TTT CGA GGC CTC
170
                          Met Ser Val Ala Ala Val Phe Arg Gly Leu
                          -39             -35                 -30

CGG CCA AGT CCT GAG CTG CTG CTT CTG GGC CTG TTG TTT CTG CCA GCG
218
Arg Pro Ser Pro Glu Leu Leu Leu Leu Gly Leu Leu Phe Leu Pro Ala
                  -25                 -20                 -15

GTG GTT GCT GTC ACC AGC GCT GGT CCC GAA GAA AGC GAT GGA GAT CTT
266
Val Val Ala Val Thr Ser Ala Gly Pro Glu Glu Ser Asp Gly Asp Leu
             -10                 -5                  1

AGC TGT GTG TGT GTG AAG ACC ATC TCC TCT GGG ATC CAT CTT AAG CAC
314
Ser Cys Val Cys Val Lys Thr Ile Ser Ser Gly Ile His Leu Lys His
      5                  10                  15

ATC ACC AGC CTG GAG GTG ATC AAG GCA GGA CGC CAC TGT GCG GTT CCC
362
Ile Thr Ser Leu Glu Val Ile Lys Ala Gly Arg His Cys Ala Val Pro
 20                  25                  30                  35

CAG CTC ATA GCC ACC CTG AAG AAT GGG AGG AAA ATT TGC CTG GAC CGG
410
Gln Leu Ile Ala Thr Leu Lys Asn Gly Arg Lys Ile Cys Leu Asp Arg
                  40                  45                  50

CAA GCA CCC CTA TAT AAG AAA GTA ATC AAG AAA ATC CTG GAG AGT
455
Gln Ala Pro Leu Tyr Lys Lys Val Ile Lys Lys Ile Leu Glu Ser
             55                  60                  65
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TAGGTATCAG CTGCCTAAAT GTCAATTGTG TTACAAGACT CCTGGAATCT TGTCTACTTT
515

TAATGTAACT GCAATCTTCC GATGT
540

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 105 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Ser Val Ala Ala Val Phe Arg Gly Leu Arg Pro Ser Pro Glu Leu
-39             -35                 -30                 -25

Leu Leu Leu Gly Leu Leu Phe Leu Pro Ala Val Val Ala Val Thr Ser
            -20                 -15                 -10

Ala Gly Pro Glu Glu Ser Asp Gly Asp Leu Ser Cys Val Cys Val Lys
         -5                  1                   5

Thr Ile Ser Ser Gly Ile His Leu Lys His Ile Thr Ser Leu Glu Val
 10                  15                  20                  25

Ile Lys Ala Gly Arg His Cys Ala Val Pro Gln Leu Ile Ala Thr Leu
                30                  35                  40

Lys Asn Gly Arg Lys Ile Cys Leu Asp Arg Gln Ala Pro Leu Tyr Lys
            45                  50                  55

Lys Val Ile Lys Lys Ile Leu Glu Ser
        60                  65
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 442 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 74..412

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 200..412

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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60

ACTGCTCTTC ATT ATG GGC TTC AGA CTC AGA CCT ACA TCG TCC TGC ACC
109
                Met Gly Phe Arg Leu Arg Pro Thr Ser Ser Cys Thr
                -42     -40                 -35

AGG GCC TGC CCA CTT CAT AAC CTC CAG ATC TTG CTG CTG CTG GGC CTG
157
Arg Ala Cys Pro Leu His Asn Leu Gln Ile Leu Leu Leu Leu Gly Leu
-30                 -25                 -20                 -15

ATC CTT GTT GCG CTG GCT CCC CTT ACA GCT GGA AAA TCT GAT GGC ATG
205
Ile Leu Val Ala Leu Ala Pro Leu Thr Ala Gly Lys Ser Asp Gly Met
                -10                 -5                  1

GAC CCA TAT ATC GAA CTG CGC TGC AGA TGT ACG AAT ACC ATC TCT GGA
253
Asp Pro Tyr Ile Glu Leu Arg Cys Arg Cys Thr Asn Thr Ile Ser Gly
          5                  10                  15

ATC CCA TTC AAT TCT ATC TCC CTT GTG AAT GTG TAC AGG CCA GGA GTT
301
Ile Pro Phe Asn Ser Ile Ser Leu Val Asn Val Tyr Arg Pro Gly Val
     20                  25                  30

CAC TGT GCT GAT GTG GAA GTG ATA GCC ACA CTG AAG AAT GGA CAA AAA
349
His Cys Ala Asp Val Glu Val Ile Ala Thr Leu Lys Asn Gly Gln Lys
 35                  40                  45                  50

ACG TGC CTG GAC CCA AAT GCC CCT GGC GTC AAG AGA ATC GTC ATG AAA
397
Thr Cys Leu Asp Pro Asn Ala Pro Gly Val Lys Arg Ile Val Met Lys
                55                  60                  65

ATC TTG GAA GGT TAC TGACCAGCTG CTTCATCTGT GCCAAACCAT
442
Ile Leu Glu Gly Tyr
             70
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 113 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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-42     -40                 -35                 -30

Leu His Asn Leu Gln Ile Leu Leu Leu Leu Gly Leu Ile Leu Val Ala
    -25                 -20                 -15

Leu Ala Pro Leu Thr Ala Gly Lys Ser Asp Gly Met Asp Pro Tyr Ile
-10                 -5                  1                   5
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Glu Leu Arg Cys Arg Cys Thr Asn Thr Ile Ser Gly Ile Pro Phe Asn
10 15 20

Ser Ile Ser Leu Val Asn Val Tyr Arg Pro Gly Val His Cys Ala Asp
25 30 35

Val Glu Val Ile Ala Thr Leu Lys Asn Gly Gln Lys Thr Cys Leu Asp
40 45 50

Pro Asn Ala Pro Gly Val Lys Arg Ile Val Met Lys Ile Leu Glu Gly
55 60 65 70

Tyr

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 893 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 71..469

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 140..469

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATTCGGATCC ATCCTTGCGG CTGTCCATCT CACCTACAGC TCTGGTCTCA TCCTCAACTC
60

AACCACAATC ATG GCT CAG ATG ATG ACT CTG AGC CTC CTT AGC CTG GTC
109
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-23 -20 -15

CTG GCT CTC TGC ATC CCC TGG ACC CAA GGC AGT GAT GGA GGG GGT CAG
157
Leu Ala Leu Cys Ile Pro Trp Thr Gln Gly Ser Asp Gly Gly Gly Gln
-10 -5 1 5

GAC TGC TGC CTT AAG TAC AGC CAG AAG AAA ATT CCC TAC AGT ATT GTC
205
Asp Cys Cys Leu Lys Tyr Ser Gln Lys Lys Ile Pro Tyr Ser Ile Val
10 15 20

CGA GGC TAT AGG AAG CAA GAA CCA AGT TTA GGC TGT CCC ATC CCG GCA
253
Arg Gly Tyr Arg Lys Gln Glu Pro Ser Leu Gly Cys Pro Ile Pro Ala
25 30 35

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ATC CTG TTC TCA CCC CGG AAG CAC TCT AAG CCT GAG CTA TGT GCA AAC
301
Ile Leu Phe Ser Pro Arg Lys His Ser Lys Pro Glu Leu Cys Ala Asn
     40                  45                  50

CCT GAG GAA GGC TGG GTG CAG AAC CTG ATG CGC CGC CTG GAC CAG CCT
349
Pro Glu Glu Gly Trp Val Gln Asn Leu Met Arg Arg Leu Asp Gln Pro
 55                  60                  65                  70

CCA GCC CCA GGG AAA CAA AGC CCC GGC TGC AGG AAG AAC CGG GGA ACC
397
Pro Ala Pro Gly Lys Gln Ser Pro Gly Cys Arg Lys Asn Arg Gly Thr
                 75                  80                  85

TCT AAG TCT GGA AAG AAA GGA AAG GGC TCC AAG GGC TGC AAG AGA ACT
445
Ser Lys Ser Gly Lys Lys Gly Lys Gly Ser Lys Gly Cys Lys Arg Thr
             90                  95                 100

GAA CAG ACA CAG CCC TCA AGA GGA TAGCCCAGTA GCCCGCCTGG AGCCCAGGAG
499
Glu Gln Thr Gln Pro Ser Arg Gly
        105                 110

ATCCCCCACG AACTTCAAGC TGGGTGGTTC ACGGTCCAAC TCACAGGCAA AGAGGGAGCT
559

AGAAAACAGA CTCAGGAGCC CAAAGCAGCC ACCTCATGCT GGCCTCCGTC CACACCCTTG
619

CCCTGCTTCA ACCATTACAT TTGCACGGCC ATCCCTTTTT TACCTGGCGG AGCTGCCTTC
679

CCCTGGGGTA GACCTAGAGA GTCAGAAGAA AGAGTGTTTC CCAGGGAATG AGGAAGGAGA
739

CAGCAGGACT GTCCCCTCTA GGAGGTCACT CAGGTCCCAA GACCTGAACC TGTTTTCCAT
799

GGCGCCCTTC CCTTGTCCTT GCACCTATGA TTTATACCTA ACTGAATAAA AAGGTGATCC
859

AGCCTCAAAA AAAAAAAAAA AAAAAAAAAA AAAA
893
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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 133 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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Met Ala Gln Met Met Thr Leu Ser Leu Leu Ser Leu Val Leu Ala Leu
-23          -20                 -15                 -10

Cys Ile Pro Trp Thr Gln Gly Ser Asp Gly Gly Gly Gln Asp Cys Cys
          -5                  1                   5
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Leu Lys Tyr Ser Gln Lys Lys Ile Pro Tyr Ser Ile Val Arg Gly Tyr
10 15 20 25

Arg Lys Gln Glu Pro Ser Leu Gly Cys Pro Ile Pro Ala Ile Leu Phe
30 35 40

Ser Pro Arg Lys His Ser Lys Pro Glu Leu Cys Ala Asn Pro Glu Glu
45 50 55

Gly Trp Val Gln Asn Leu Met Arg Arg Leu Asp Gln Pro Pro Ala Pro
60 65 70

Gly Lys Gln Ser Pro Gly Cys Arg Lys Asn Arg Gly Thr Ser Lys Ser
75 80 85

Gly Lys Lys Gly Lys Gly Ser Lys Gly Cys Lys Arg Thr Glu Gln Thr
90 95 100 105

Gln Pro Ser Arg Gly
110

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 814 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 6..407

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 75..407

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAGAC ATG GCT CAG TCA CTG GCT CTG AGC CTC CTT ATC CTG GTT CTG
47
Met Ala Gln Ser Leu Ala Leu Ser Leu Leu Ile Leu Val Leu
-23 -20 -15 -10

GCC TTT GGA ATC CCC AGG ACC CAA GGC AGT GAT GGA GGG GCT CAG GAC
95
Ala Phe Gly Ile Pro Arg Thr Gln Gly Ser Asp Gly Gly Ala Gln Asp
-5 1 5

TGT TGC CTC AAG TAC AGC CAA AGG AAG ATT CCC GCC AAG GTT GTC CGC
143
Cys Cys Leu Lys Tyr Ser Gln Arg Lys Ile Pro Ala Lys Val Val Arg
10 15 20

AGC TAC CGG AAG CAG GAA CCA AGC TTA GGC TGC TCC ATC CCA GCT ATC
191
Ser Tyr Arg Lys Gln Glu Pro Ser Leu Gly Cys Ser Ile Pro Ala Ile
25 30 35

CTG TTC TTG CCC CGC AAG CGC TCT CAG GCA GAG CTA TGT GCA GAC CCA
239
Leu Phe Leu Pro Arg Lys Arg Ser Gln Ala Glu Leu Cys Ala Asp Pro
40 45 50 55

AAG GAG CTC TGG GTG CAG CAG CTG ATG CAG CAT CTG GAC AAG ACA CCA
287
Lys Glu Leu Trp Val Gln Gln Leu Met Gln His Leu Asp Lys Thr Pro
60 65 70

TCC CCA CAG AAA CCA GCC CAG GGC TGC AGG AAG GAC AGG GGG GCC TCC
335
Ser Pro Gln Lys Pro Ala Gln Gly Cys Arg Lys Asp Arg Gly Ala Ser
75 80 85

AAG ACT GGC AAG AAA GGA AAG GGC TCC AAA GGC TGC AAG AGG ACT GAG
383
Lys Thr Gly Lys Lys Gly Lys Gly Ser Lys Gly Cys Lys Arg Thr Glu
90 95 100

CGG TCA CAG ACC CCT AAA GGG CCA TAGCCCAGTG AGCAGCCTGG AGCCCTGGAG
437
Arg Ser Gln Thr Pro Lys Gly Pro
105 110

ACCCCACCAG CTTCACCAGC GCTTGAAGCC TGAACCCAAG ATGCAAGAAG GAGGCTATGC
497

TCAGGGGCCC TGGAGCAGCC ACCCCATGCT GGCCTTGCCA CACTCTTTCT CCTGCTTTAA
557

CCACCCCATC TGCATTCCCA GCTCTACCCT GCATGGCTGA GCTGCCCACA GCAGGCCAGG
617

TCCAGAGAGA CCGAGGAGGG AGAGTCTCCC AGGGAGCATG AGAGGAGGCA GCAGGACTGT
677

CCCCTTGAAG GAGAATCATC AGGACCCTGG ACCTGATACG GCTCCCCAGT ACACCCCACC
737

TCTTCCTTGT AAATATGATT TATACCTAAC TGAATAAAAA GCTGTTCTGT CTTCCCACCC
797

AAAAAAAAAA AAAAAAA
814

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 134 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Ala Gln Ser Leu Ala Leu Ser Leu Leu Ile Leu Val Leu Ala Phe
-23          -20                -15                -10

Gly Ile Pro Arg Thr Gln Gly Ser Asp Gly Gly Ala Gln Asp Cys Cys
          -5                  1                   5

Leu Lys Tyr Ser Gln Arg Lys Ile Pro Ala Lys Val Val Arg Ser Tyr
 10                  15                  20                  25

Arg Lys Gln Glu Pro Ser Leu Gly Cys Ser Ile Pro Ala Ile Leu Phe
                    30                  35                  40

Leu Pro Arg Lys Arg Ser Gln Ala Glu Leu Cys Ala Asp Pro Lys Glu
                45                  50                  55

Leu Trp Val Gln Gln Leu Met Gln His Leu Asp Lys Thr Pro Ser Pro
            60                  65                  70

Gln Lys Pro Ala Gln Gly Cys Arg Lys Asp Arg Gly Ala Ser Lys Thr
        75                  80                  85

Gly Lys Lys Gly Lys Gly Ser Lys Gly Cys Lys Arg Thr Glu Arg Ser
 90                  95                  100                 105

Gln Thr Pro Lys Gly Pro
                110
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 24..77
(D) OTHER INFORMATION: /note= "mature peptide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Met Xaa Gln Ser Leu Val Leu Arg Ile Leu Val Leu Val Leu Ala Phe
1               5                  10                  15

Cys Ile Pro His Thr Gln Gly Ser Asp Gly Gly Ala Gln Asp Cys Cys
            20                  25                  30

Leu Lys Tyr Ser Leu Arg Lys Ile Pro Thr His Val Val Arg Ser Tyr
        35                  40                  45

Arg Lys Gln Glu Pro Ser Leu Gly Cys Pro Ile Pro Ala Ile Leu Phe
    50                  55                  60

Ser Pro Arg Lys Xaa Ser Gln Pro Glu Leu Cys Ala Asp
65                  70                  75
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Arg Phe Glu Gly Tyr Arg Pro Asn Leu Ala Glu Ser Cys Pro Cys Arg
1 5 10 15

Gln Tyr Arg Ala Tyr Val Leu Thr His Ser Gly Glu Leu Tyr Glu Arg
20 25 30

Pro Glu Arg Ser Asp Arg Gln Ile Cys Val
35 40

WHAT IS CLAIMED IS:

1. A substantially pure or recombinant polypeptide which:

(a) comprises a plurality of epitopes found on; and

(b) exhibits at least 85% sequence identity over a length of at least 12 contiguous amino acids to;

a polypeptide selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 10.

2. The polypeptide of Claim 1, wherein the polypeptide binds with specificity to an antibody generated against an immunogen selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 10.

3. A fusion protein comprising a polypeptide according to either Claim 1 or 2.

4. An isolated nucleic acid which encodes a polypeptide or fusion protein of any of Claims 1-3.

5. The nucleic acid of Claim 4, selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 7.

6. A nucleic acid which:

a) hybridizes under wash conditions of 30° C and less than 2M salt to a nucleic acid selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 7; or

b) exhibits at least about 85% identity over a stretch of at least about 30 nucleotides to a nucleic acid selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 7.

7. A vector comprising a nucleic acid of any of Claims 4-6.

8. A host cell comprising a nucleic acid or vector of any of Claims 4-7.

9. A method for making a polypeptide or fusion protein comprising culturing a host cell of Claim 8 under conditions in which the nucleic acid or vector is expressed.

10. A binding compound comprising an antibody or antigen binding fragment therefrom which binds with specificity to a polypeptide of either Claim 1 or 2.

11. A composition comprising a polypeptide or fusion protein according to any one of Claims 1-3.

12. A kit comprising:

a) a protein, polypeptide or fusion protein according to any one of Claims 1 to 3;

b) an antibody which specifically binds to a protein or peptide according to either Claim 1 or 2; or

c) a nucleic acid according to any one of Claims 4-6.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/17122

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/19 C07K14/52 C07K16/24 A61K38/19 C12Q1/68
G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 97 07198 A (GENETICS INSTITUTE, INC.) 27 February 1997 see SEQ ID NO:5 and NO:6 see claims 21-30 ---	1,2,4-9, 11
X	DOI T. ET AL.: "Structure of the rat platelet factor 4 gene: a marker for megakaryocyte differentiation" MOLECULAR AND CELLULAR BIOLOGY, vol. 7, 1987, pages 898-904, XP002054439 cited in the application	1,2,4-8
Y	see figure 4 --- -/--	3

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

3

Date of the actual completion of the international search	Date of mailing of the international search report
5 February 1998	13. 03. 98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Olsen, L

INTERNATIONAL SEARCH REPORT

Interr ·al Application No
PCT/US 97/17122

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PONCZ M.R. ET AL.: "Cloning and characterization of platelet factor 4 cDNA derived from a human erythroleukemic cell line" BLOOD, vol. 69, 1987, pages 219-223, XP002054440	1,2,4-8
Y	see figure 1	3

X	DATABASE EMBL, EMEST7 Entry Mma14819, Accession number AA014819 "The WashU-HHMI Mouse EST Project". Unpublished, 3 August 1996 XP002054441 see abstract	4-6

X	WO 96 13587 A (REPLIGEN CORP) 9 May 1996	1,2,4-8
Y	see claims 1-34; figure 3A	3

X	WO 94 12537 A (REGA INSTITUTE LEUVEN UNIVERSITY) 9 June 1994	1,2,4-8
Y	see SEQ ID NO:40	3

X	DATABASE EMBL, EMEST7 Entry Mm80724, Accession number W53807 "The WashU-HHMI Mouse EST Project". Unpublished., 6 June 1996 XP002054442 cited in the application see abstract	4-6

X	WO 95 35376 A (CHIRON CORP) 28 December 1995	1,2
Y	see SEQ ID NO:2	3

X	WO 96 06169 A (HUMAN GENOME SCIENCES INC.) 29 February 1996	1,2,4-9, 11,12
Y	see SEQ ID NO:1 and NO:2 see claims 1-18	3

X	WO 96 25497 A (INCYTE PHARMACEUTICALS, INC.) 22 August 1996	1,2,4-12
Y	see SEQ ID NO:3 and NO:4 see claims 13-24; figure 2	3

X	DATABASE EMBL, EMEST7 Entry Mm04637, Accession number W67046 "The WashU-HHMI Mouse EST Project". Unpublished., 15 June 1996 XP002054443 cited in the application see abstract	4-6

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INTERNATIONAL SEARCH REPORT

Interr. ıal Application No
PCT/US 97/17122

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL, EMEST7 Entry Hsw84422, Accession number W84422 "The WashU-Merck EST Project". Unpublished., 1 July 1996 XP002054444 see abstract ---	4-6
X	DATABASE EMBL, EMEST3 Entry Hs024168, Accession number H14024 "Map: Chromosome19p12-p13.1". Unpublished., 3 July 1995 XP002054445 see abstract ---	1,2
A	KELNER G S ET AL: "LYMPHOTACTIN: A CYTOKINE THAT REPRESENTS A NEW CLASS OF CHEMOKINE" SCIENCE, vol. 266, no. 5189, 25 November 1994, pages 1395-1399, XP002047642 cited in the application see the whole document ---	1-12
A	LODI P.J. ET AL.: "HIGH-RESOLUTION SOLUTION STRUCTURE OF THE BETA CHEMOKINE hMIP-1-BETA BY MULTIDIMENSIONAL NMR" SCIENCE, vol. 263, 25 March 1994, pages 1762-1767, XP002054455 cited in the application see the whole document -----	1-12

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr nal Application No
PCT/US 97/17122

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9707198 A	27-02-97	US 5707829 A	13-01-98
		AU 6712396 A	18-02-97
		AU 6768596 A	12-03-97
		WO 9704097 A	06-02-97
WO 9613587 A	09-05-96	US 5656724 A	12-08-97
		AU 4013495 A	23-05-96
		CA 2202889 A	09-05-96
		EP 0786001 A	30-07-97
WO 9412537 A	09-06-94	AU 5628394 A	22-06-94
		EP 0804486 A	05-11-97
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		CA 2193526 A	28-12-95
		EP 0766737 A	09-04-97
WO 9606169 A	29-02-96	AU 2814195 A	14-03-96
		CA 2198206 A	29-02-96
		EP 0799311 A	08-10-97
WO 9625497 A	22-08-96	AU 5024696 A	04-09-96
		CA 2209227 A	22-08-96
		EP 0809699 A	03-12-97